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The overall objective of this project was to determine the neural factors which are involved in the regulation of the cardiovascular system during various stresses. To achieve these objectives, we have investigated factors which influence the control of heart rate under normal laboratory conditions as well as during stresses such as myocardial ischemia, acute volume expansion or depletion and during exposure to positive acceleration. Our second objective was to establish the contribution of neural reflexes originating from receptors located in the cardiopulmonary and sinoaortic regions on the beat-to-beat control of the heart and the peripheral circulation under various laboratory stresses and during the stress of positive acceleration. The detailed report is broken down into sections. Each section is a separate phase of the overall study.

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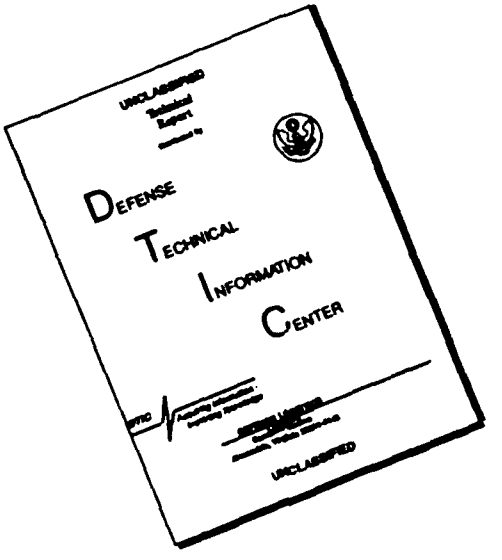

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OBJECTIVE: The overall objective of this project was to determine the neural factors which are involved in the regulation of the cardiovascular system during various stresses. To achieve these objectives, we have investigated factors which influence the control of heart rate under normal laboratory conditions as well as during stresses such as myocardial ischemia, acute volume expansion or depletion and during exposure to positive acceleration. Our second objective was to establish the contribution of neural reflexes originating from receptors located in the cardiopulmonary and sinoaortic regions on the beat-to-beat control of the heart and the peripheral circulation under various laboratory stresses and during the stress of positive acceleration.

This detailed report is broken down into sections. Each section is a separate phase of the overall study. They are as follows:

1. FACTORS INFLUENCING THE INTRINSIC CONTROL OF HEART RATE.

A. Interaction of Acetylcholine and Norepinephrine on Heart Rate

Responses of isolated rat atria to acetylcholine (Ach) indicate that onset of bradycardia occurs when concentration in the bathing solution reaches 10^{-8} molar. The degree of slowing is directly proportional to the concentration of Ach. All hearts stopped or nearly stopped when Ach concentration reached 10^{-4} M. Norepinephrine produced tachycardia. Onset occurred at 10^{-8} M and peak response occurred at 10^{-5} M. The dose-response curves established the control values against which other comparisons could be made.

When norepinephrine was added to the bathing solution prior to establishing a dose-response curve for acetylcholine, it had little influence on the response of the atrium to acetylcholine at concentrations of 10^{-9} or 10^{-7} M. At concentrations of 10^{-5} M of norepinephrine, the threshold of responsiveness to Ach was higher as was the ED_{50}, although the peak response was similar to the control value. This indicates that only at high doses of norepinephrine are low doses of Ach interfered with. High doses of Ach were still not influenced by prior tissue exposure to high doses of epinephrine.

When acetylcholine was added to the bathing solution prior to establishing a dose-response curve for norepinephrine, a definite influence was observed when the concentration reached 10^{-7} M. The same was true for 10^{-5} M Ach. The peak responses were also reduced at these concentrations. Thus, acetylcholine definitely inhibits both the onset and magnitude of responses to norepinephrine.

B. Experimental Heart Failure Effects on Atrial Response to Norepinephrine

Chronotropic and inotropic responses to norepinephrine (NE) (10^{-13} - 10^{-5} M) were obtained for isolated rat atria. Eleven to seventeen days

postoperatively, tissue was taken from 14 sham-operated controls and 16 rats with experimental heart failure produced by aortic constriction. Presacrificial heart rates were higher in constricted animals ($P < .01$) but not different from shams when isolated in the tissue bath. Chronotropic change was observed in the heart failure group at 10^{-13} M NE, while significant change in the control group was seen at 10^{-8} M NE. The maximum heart rate changes for NE occurred with 10^{-5} M for both groups. Positive inotropic changes were observed in the heart failure group at 10^{-13} M NE, while the earliest change in the control group was at 10^{-9} M NE. The maximum inotropic response for both groups occurred at 10^{-6} M NE. ED_{50} for the two groups was not significantly different. These findings show that rat atria are more responsive to low levels of NE after heart failure than are controls. This is consistent with observations that note a defect in uptake and storage of NE in this type of experimental congestive heart failure. If such an abnormality makes increased quantities of NE available at the receptor site at low circulating NE levels, individuals in congestive heart failure might be hyper-responsive to normal blood NE levels.

2. BEAT-TO-BEAT REGULATION OF HEART RATE BY AFFERENT STIMULATION OF THE AORTIC NERVE. (See Appendix Reprint #1)

Repeated electrical stimulation of the aortic nerve, when confined to one cardiac cycle, caused heart rate reduction in the anesthetized rabbit. The average fall in heart rate due to supramaximal stimulation was 8.4 ± 0.3 beats/min ($\pm$ SEM). Extent of bradycardia was more closely related to total number of impulses within the stimulus burst than to either burst duration or impulse frequency. The latency to onset of the response could not be altered by changes in any of the stimulus para-

meters, nor could it be related to the position of the stimulus burst within the cardiac cycle. These results indicate that beat-to-beat regulation of heart rate can be accomplished when afferent aortic activity is altered.

3. LEFT VENTRICULAR FUNCTION DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA. (See Appendix Reprint #2)

The effects of acute 1-min. occlusion of the left circumflex coronary artery on the inotropic state and performance of the left ventricle were examined in adult mongrel dogs. The inotropic state, as indicated by changes in the maximum derivative of left ventricular pressure in the pre-ejection phase and the maximum derivative of the transverse internal diameter, were diminished during the ischemic period. The end-systolic diameter increased 3.8 $\pm$ 0.6 mm while the end-diastolic diameter increased only 0.4 $\pm$ 0.2 mm, although the end-diastolic pressure increased 6.9 $\pm$ 0.6 mmHg. Progressive decreases in the stroke volume paralleled the apparent reduction in myocardial fiber shortening in the transverse plane. Cardiac output and arterial pressure declined, concurrently, thus maintaining a constant peripheral increase during the occlusion. Acute coronary occlusion also caused an apparent increase in the myocardial wall stiffness, as judged by the increase in the slope a of the equation $dP/dV = aP + B$, the increase of the slope of the pressure-diameter relationship, and the decrease in the rate of lengthening of the diameter during diastole. These findings suggest that acute myocardial ischemia results in an immediate reduction in the effective inotropic state and an apparent increase in the myocardial wall stiffness in the transverse plane. Both of these changes resulted in a decrease in performance of the left ventricle.

4. SPECIFICITY OF AUTONOMIC INFLUENCES ON CARDIAC RESPONSES DURING MYOCARDIAL ISCHEMIA. (See Appendix Reprint #3)

A possible role of the autonomic nervous system in the left ventricular response to acute regional myocardial ischemia was sought in conscious dogs instrumented for measurement of left ventricular pressure, internal diameter, and aortic flow. Ischemia produced by occluding the left circumflex coronary artery caused tachycardia and reduced contractility. Changes during control occlusions were compared with those during occlusion after beta-adrenergic blockade, parasympathetic blockade, and combined sympathetic and parasympathetic blockade. Beta-blockade did reduce the tachycardia and slightly reduced left ventricular diameter changes in response to coronary occlusion. Results obtained in animals following surgical cardiac sympathectomy indicated reduced tachycardia and no effects on other parameters. The principal effect of parasympathetic blockade was to augment the increase in end-diastolic diameter during occlusion. Right atrial pacing indicated this change was due to higher initial heart rates. Combined parasympathetic and sympathetic blockade did not alter inotropic responses to coronary occlusion. Results indicated that inotropic support due to changes in activity in autonomic nerves is not increased during acute occlusion of the left circumflex coronary artery.

5. REFLEX HEART RATE CONTROL VIA SPECIFIC AORTIC NERVE AFFERENTS IN THE RABBIT. (See Appendix Reprint #4)

Reflex bradycardia was elicited in rabbits via repetitive electrical stimulation of the central end of the sectioned left aortic nerve. Supramaximal stimulation produced 16.9 $\pm$ 1.3% (SE) increase in the R-R interval when vagal and sympathetic efferent pathways were intact. Reducing the stimulation voltage allowed selective stimulation of the myelinated (A) fibers, and polarizing electrodes placed central to the stimulus site per-

mitted A fiber blockade and selective stimulation of the unmyelinated (C) fibers. When afferent A fibers were selectively stimulated, 64% of the maximum response was obtained; selective C fiber activation elicited 63% of the maximum observed response. Selective stimulation of A or C fibers after either vagotomy or stellectomy indicated that A fiber afferents elicit heart rate responses via both vagal and sympathetic efferents, whereas C fiber afferent information is mediated predominantly via vagal efferents. This afferent-efferent specificity of the aortic baroreceptor pathways suggest baroreceptor mechanisms normally used to modulate heart rate. Small increments in blood pressure would activate low-threshold A fibers and result in reciprocal changes in vagal and sympathetic efferent activity. More substantial increases in blood pressure would activate afferent C fibers and produce additional heart rate effects via vagal efferents.

6. CARDIOVASCULAR CHANGES DURING AND FOLLOWING 1-MIN EXPOSURE TO $+G_Z$ STRESS. (See Appendix Reprint #5)

Magnitude and duration of cardiovascular responses following $+G_Z$ forces of 1 - 5 G were studied in chronically instrumented anesthetized dogs. During lower G forces (+1 to $+3G_Z$), responses were variable. In most dogs during higher G forces (+4 or +5 G_Z), aortic pressure, cardiac output, left ventricular pressure, and dP/dt were all dramatically compromised. These changes were observed whether the onset of the gravitational inertial force was slow (0.1 G/s) or rapid (1.0 G/s). Cardiovascular changes after acceleration were consistent. Left atrial pressure and arterial pressure rose and a transient rise in dP/dt was often observed. Cardiac output rose briefly, then fell; hence, peripheral resistance increased. Magnitude and duration of these changes were directly

related to G forces during acceleration. Our results confirm that $+G_Z$ stress produces major cardiovascular changes. Our experiments also demonstrate that responses following $+G_Z$ stress may be dramatic and prolonged. Increased peripheral resistance elevates perfusion pressure and, concurrently, the increased preload may cause acute cardiopulmonary congestion.

7. THE ROLE OF NEURAL FACTORS IN THE CARDIOVASCULAR RESPONSE TO ACUTE VOLUME LOADING. (See Appendix Reprint #6)

The influence of the cardiac sympathetic nerves (CSN) and arterial baroreceptors on the cardiovascular responses to acute volume loading (AVL) was investigated in 20 conscious dogs. All animals were previously instrumented with electromagnetic flow probes for the measurement of cardiac output (CO), catheters for measuring left atrial pressure (LAP) and arterial pressure (AP). AVL increased LAP (15 mmHg), CO (+1439 cc/min), HR (28 b/min) and AP (13 mmHg) while decreasing peripheral resistance (PR) (-0.87 PRU, 37%). In all 5 animals, baroreceptor denervation did not alter the above responses to volume loading. Surgical section of the sympathetic innervation to the heart, in 6 animals, significantly reduced the ΔHR to volume loading (35 to 21 b/min) and, consequently the CO was less (1863 to 977 cc/min). Since the AP response was unaltered, the decline in PR to volume loading was significantly less (-0.52 PRU as compared to -0.88 PRU). In 5 animals, selective removal of the left CSN had no effect on the responses to AVL, while in other animals, initial removal of the right CSN significantly reduced ΔHR from 35 to 17 b/min. Vagal blockade resulted in a fall or no change in HR, during AVL.

However, a small positive ΔHR response to AVL was observed after combined vagal blockade and bilateral cardiac sympathectomy. Epinephrine infusion augmented the ΔHR response to AVL with or without cardiac sympathetic innervation. These observations suggest the ΔHR is mediated via the vagus and the magnitude is modulated by the cardiac sympathetic nerves.

8. CARDIOVASCULAR RESPONSES TO ELECTROCARDIOGRAM-COUPLED STIMULATION OF RABBIT AORTIC NERVE. (See Appendix Reprint #7)

Electrical stimulation of the rabbit's aortic nerve during one or more cardiac cycles resulted in a reflex fall in heart rate and mean arterial pressure (MAP). The onset of bradycardia and of fall in MAP were independent of the number of beats stimulated. The initial slope of the heart rate and MAP responses increased as the number of beats stimulated increased, reaching a maximum at five beats of stimulation. Bradycardia peaked 8 and 10 beats after the end of one and two cycles of stimulation, respectively, while the peak response occurred at, or prior to, the end of stimulation when 12 or more beats were involved. Onset and recovery of both responses were consistent, and seldom did MAP indicate a return toward control during stimulation, Thus, central nervous system modulation of sympathetic activity to the peripheral vasculature was sustained as long as the aortic nerve input was maintained. However, reflex control of heart rate was more complex, involving simultaneous alteration in both vagal and sympathetic efferent activity.

9. REDUCTION IN BAROREFLEX CARDIOVASCULAR RESPONSES DUE TO VENOUS INFUSION IN THE RABBIT. (See Appendix Reprint #8)

We studied reflex bradycardia and depression of mean arterial blood pressure (MAP) during left aortic nerve (LAN) stimulation before and after volume infusion in the anesthetized rabbit. Step increases in mean right atrial pressure (MRAP) to 10 mmHg did not result in a significant change in

heart rate or MAP. After volume loading, responses to LAN stimulation were not as great and the degree of attenuation was proportional to the level of increased MRAP. A change in responsiveness was observed after elevation of MRAP by only 1 mmHg, corresponding to less than a 10% increase in average calculated blood volume. After an increase in MRAP of 10 mmHg, peak responses were attenuated by 44% (heart rate) and 52% (MAP), and the initial slopes (rate of change) were reduced by 46% (heart rate) and 66% (MAP). Comparison of the responses after infusion with blood and dextran solutions indicated that hemodilution was an unlikely explanation for the attenuation of the reflex responses. Total arterial baroreceptor denervation (ABD) abolished the volume-related attenuation of the cardiovascular responses, whereas attenuation was still present following bilateral aortic nerve section or vagotomy. It thus appears that the carotid sinus responds to changes in blood volume and influences the reflex cardiovascular responses to afferent stimulation of the LAN. On the other hand, cardiopulmonary receptors subserved by vagal afferents do not appear to be involved.

10. HEMODYNAMIC RESPONSES TO CORONARY OCCLUSION IN EXERCISING DOGS (See Appendix Reprint #9)

Exercise (EX) induces increased left ventricular function whereas coronary occlusion depresses the heart. Their combined stress effects on cardiac dynamics are unknown. Seven mongrel dogs were trained to run on a level treadmill and then surgically instrumented to record left ventricular pressure, thereby permitting evaluation of systolic (LVSP) and end-diastolic (LVEDP) pressures, the maximum derivative (dP/dt) and heart rate (HR). Aortic flow probes were implanted to yield stroke volume (SV) and cardiac output (CO). Cuff occluders were placed around the left circumflex coronary artery. After full recovery, responses to coronary occlusion (Occ)

at rest and during EX, 6-8 mph, were compared. Measurements were taken approximately 3 minutes after onset of EX and 50 seconds after onset of Occ. During EX, all control values were elevated above resting controls: i.e., HR (96-196 b/min); SV (32.8-33.9 ml/b); CO (3.39-7.03 l/min); dP/dt (3171-4751 mmHg/s); LVSP (123-161 mmHg); LVEDP (3.6-6.8 mmHg). Occ caused further increases in HR and LVEDP at rest and during EX but produced decreases in all other parameters measured. Changes due to Occ were parallel in all cases except HR and CO when rest and EX were compared. Tachycardia due to resting Occ was significantly greater than that observed due to Occ during EX (29 vs 9 b/min). The fall in CO during EX occlusion was significantly greater than the small fall in CO due to resting occlusion (1.32 vs 0.13 l/min). It is apparent that the effects of Occ on myocardial function at rest and during moderate EX are similar; however, CO is compromised by ischemia during EX since increases in heart rate no longer compensate for the fall in stroke volume.

11. CARDIOVASCULAR PROTECTION WITH AN ANTI-G SUIT DURING SUSTAINED $+G_Z$ STRESS. (See Appendix Reprint #10)

Lightly anesthetized dogs underwent one minute exposure to $+G_Z$ acceleration both with and without a bladder type anti-G suit. Prior chronic instrumentation permitted evaluation of left ventricular internal diameter, heart rate aortic arch pressure, left ventricular pressure, left ventricular and diastolic pressure, left ventricular dP/dt, aortic flow and total peripheral resistance. During $+3G_Z$ acceleration without the suit inflated, all dynamic parameters were depressed and transient tachycardia was observed. After acceleration ceased, all pressures and dP/dt exceeded control levels. Inflation of the anti-G suit during $+3G_Z$ acceleration eliminated the dramatic effects observed both during and after acceleration stress. During $+6G_Z$ with the anti-G suit inflated, arterial pressure and

dP/dt were maintained whereas left ventricular end-diastolic pressure and total peripheral resistance were much elevated and heart rate was depressed. At the onset of G stress, internal diameter of the heart always fell transiently. Otherwise, diameter was not significantly affected by any of the experimental conditions. The results suggest that the anti-G suit provides important maintenance of perfusion pressure at high sustained G; however, with the anti-G suit inflated, central venous pressure is dramatically elevated and heart rate significantly depressed. Thus, the beneficial effects which provide tolerance to high G are accompanied by potentially detrimental effects.

12. INFLUENCES OF SELECTIVE CARDIAC DENERVATION ON CORONARY REACTIVE HYPEREMIA IN DOGS. (See Appendix Manuscript #11)

Mongrel dogs were chronically instrumented to measure left circumflex coronary flow, arterial pressure, left atrial pressure, ECG, heart rate and in some cases, left ventricular pressure or cardiac output. A cuff-type occluder was placed distal to the coronary flow probe. Total occlusion of the left circumflex coronary artery for one minute in unsedated, resting dogs produced reactive hyperemia with an average replacement/deficit ratio of 2.63/1. In 11 dogs sympathetic influences were investigated by chronic surgical cardiac sympathectomy. Surgical section of all ansae subclaviae reduced responses from 2.61/1 to 1.67/1 ($P < .001$). Left sympathectomy alone had no effect on the replacement/deficit ratio whereas selective right sympathectomy reduced it from 2.25/1 to 1.38/1. Pharmacological blockade was used to determine beta-receptor involvemeny in the responses. In 9 intact dogs, practolol (10 mg/kg) reduced the reactive hyperemia ratio by 12% ($P<.05$). Propranolol (1 mg/kg) further reduced this ratio by 30% ($P<.001$). Our results indicate that sympathetic beta influences work primarily through the right cardiac sympathetic nerves. Also, the

magnitude of the response appears to be due, in part, to increased metabolic activity associated with myocardial β_1 receptors and heart rate increase as well as active vasodilation through β_2 receptors.

13. RABBIT CARDIOVASCULAR RESPONSES DURING VASOACTIVE DRUG INFUSION AT FIXED CAROTID PRESSURE. (See Appendix Preprint)

In anesthetized rabbits, peak reflex bradycardia (ΔHR) and depression of mean arterial blood pressure (ΔMAP) were measured during maximal central stimulation of the left aortic nerve (LANS). Responses were quantified: (i) before and during steady state changes ($\pm$ 15 mmHg) in the isolated carotid intrasinus pressure (ISP), and (ii) with ISP excluded from the circulation and maintained at a normotensive level (EP = ISP = MAP) the MAP was changed $\pm$ 20 mmHg by the infusion of either nitroglycerin (NG), lysine vasopressin (AD) or phenylephrine (PE). Results indicated that within $\pm$ 11 mmHg of EP, the change in MAP per mmHg change in ISP was 3, while ΔMAP due to LANS changed nearly double per mmHg change in ISP. Following vagotomy a small increase in MAP was seen; however, cardiovascular responses to changes in ISP or LANS were unaltered. During drug infusion with the carotid sinuses excluded from the circulation and ISP = EP, peak ΔHR and ΔMAP to LANS were independent of the direction or magnitude of the drug induced change in MAP. When carotid baroreceptors were allowed to detect the increase in MAP, the peak ΔHR and ΔMAP responses to LANS were significantly reduced. These results suggest a high degree of sensitivity of the carotid sinus baroreceptors around the animal's normotensive region and that activity from these baroreceptors can modify reflex vascular tension even in the absence of significant change in heart rate or arterial pressure.

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Beat-to-beat regulation of heart rate by afferent stimulation of the aortic nerve

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KARDON, MERRILL B., D. FRED PETERSON, AND VERNON S. BISHOP. *Beat-to-beat regulation of heart rate by afferent stimulation of the aortic nerve.* Am. J. Physiol. 227(3): 598–600. 1974.—Repeated electrical stimulation of the aortic nerve, when confined to one cardiac cycle, caused heart rate reduction in the anesthetized rabbit. The average fall in heart rate due to supramaximal stimulation was 8.4 ± 0.3 beats/min (±SEM). Extent of bradycardia was more closely related to total number of impulses within the stimulus burst than to either burst duration or impulse frequency. The latency to onset of the response could not be altered by changes in any of the stimulus parameters, nor could it be related to the position of the stimulus burst within the cardiac cycle. These results indicate that beat-to-beat regulation of heart rate can be accompished when afferent aortic nerve activity is altered.

baroreceptors

ARTERIAL BARORECEPTORS are important sensors in the reflex control of heart rate, systemic arterial pressure, and vascular resistance (3, 8, 13, 14). However, the means by which these receptors produce subtle modification of the cardiovascular system is not known. For example, no information is available to indicate whether or not changes in baroreceptor nerve activity, when confined to a single cardiac cycle, can alter cardiovascular function during subsequent beats. If such reflex control does exist, the aortic baroreceptors, because of their proximity to the outflow of the left ventricle, would likely be involved. The aortic arch normally contains chemoreceptors as well as those sensitive to changes in blood pressure. The aortic nerve in the cat and dog carries information from both receptor sources (6, 7). In addition, it is difficult to locate anatomically, often being imbedded in the cervical vagus in the dog and cat (7). We have used rabbits because the aortic nerve is separate and easily identified and is purported to carry information predominately of baroreceptor origin (5, 12). In this study, we examined the magnitude and latency of the heart rate response resulting from the stimulation of the rabbit's aortic nerve during a single R-R interval.

METHODS

Rabbits weighing 1.5–2.0 kg were anesthetized with sodium pentobarbital 30 mg/kg iv. A femoral vein was cannulated for supplemental administration of the anesthetic. Blood pressure was monitored using a Statham P23dB strain gauge connected to a catheter in the femoral artery. Needle electrodes were placed along the sternum to monitor heart rate using a Beckman 9857B cardiotachometer coupler. All parameters were recorded on a Beckman R411 oscillograph. A tracheotomy was performed and the animals were artificially ventilated to assure the maintenance of normal blood Po_2, Pco_2, and pH. The left aortic nerve (LAN) was isolated in the cervical region as previously described (11) and sectioned at a level 1 cm rostal to the point of the sternum. The central end of the aortic nerve was placed onto bipolar hook electrodes (platinum-irridium) which were connected to a Grass SD9 stimulator. A pulse from the cardiotachometer which was synchronous with each R wave of the ECG activated the Schmitt trigger of a DEC PDP 8/E digital computer. Electrical stimulation of the LAN and continuous calculation of each R-R interval were accomplished using the computer. An experimental trial consisted of 10 successive control heartbeats followed by a burst of electrical impulses (stimulus burst) inserted at a predetermined position within the next R-R interval (11th beat). The response to stimulation was monitored until a total of 25 s had elapsed. Subsequent trials were performed at 1-min intervals using the same stimulus conditions. After 10 successive trials had been summated, an average R-R interval was calculated by the computer for each heartbeat during the control state and following the stimulation. From these data, the peak changes in R-R interval as well as the latencies to onset and peak response were calculated. The latency to onset was measured as the time from the beginning of the stimulus burst to the end of the first R-R interval which exceeded the average control interval. Latency to peak response was measured as the time from the beginning of the stimulus burst to the end of the longest heart interval monitored during the summated trials.

RESULTS

Previous studies in our laboratory have shown that during continuous aortic nerve stimulation maximum changes in heart rate can be elicited by a stimulation frequency of 80 Hz using 10-V rectangular pulses of 0.3 ms duration (11). Using these parameters, we stimulated the aortic nerve during one R-R interval (10 pulses at 80 Hz) to determine if heart rate changes could be elicited. In 17 animals, the average decrease in heart rate was 8.4 ± 0.33 beats/min (±SEM) from a resting level of 287 ± 4.2 beats/min ($P < 0.001$). Following the beginning of the stimulus burst, the latencies to onset and peak response were 1.7 ± 0.1 and

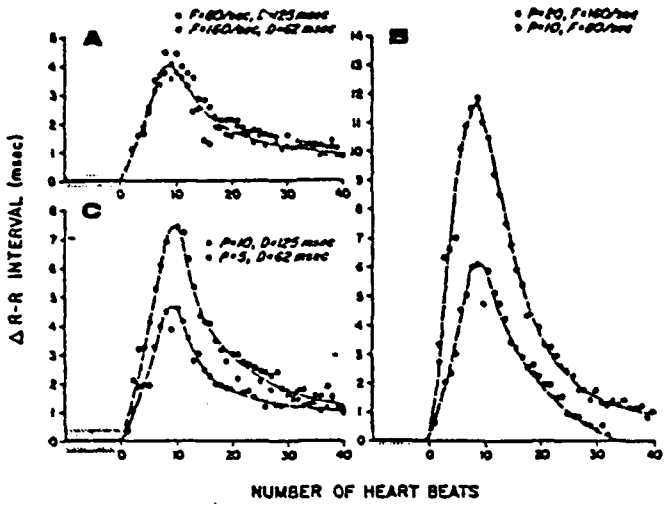

FIG. 1. Average heart interval response to left aortic nerve (LAN) stimulation. A single stimulus burst confined to 1 R-R interval is applied to LAN at heartbeat 0. Changes in R-R (heart) interval in milliseconds are indicated on ordinate. Numbers of heartbeats during a 10-beat control period (to left of zero) and during response segment (to right of zero) are shown on abscissa. *A:* average response in 8 animals when pulse number was fixed at 10. *B:* burst duration was fixed at 125 ms ($n = 6$). *C:* pulse frequency was fixed at 80 pulses/s ($n = 7$). In each case a significant difference existed at peak of response $P < .05$; however, it is apparent that difference in peak response when pulse number is fixed, *part A* is slight in comparison to conditions in *parts B* and *C*.

9.2 ± 0.3 intervals, respectively. Change in location of the stimulus burst within the R-R interval had no effect on either of the latencies or the peak response. Thus, reflex heart rate changes were initiated within one heart cycle following an alteration in aortic nerve activity during a single R-R interval.

In order to investigate the influence of each of the three variables of the stimulus burst on the heart rate response (burst duration × impulse frequency = total impulse number), each was held constant during 10 consecutive trials. Figure 1 shows the relationship between changes in R-R interval and elapsed heartbeats during a series of trials. In each panel (*A*, *B*, *C*) one of the three stimulus variables, impulse number (*A*), burst duration (*B*), or impulse frequency (*C*), was held constant. A comparison could then be made as the two remaining stimulus conditions were altered.

Figure 1*A* illustrates the reflex heart interval response (R-R interval) when the product of impulse frequency and burst duration (impulse number) was held constant at 10. The response elicited by a stimulus burst duration of 125 ms was compared to that elicited by a 62-ms burst. Thus, impulse frequency was simultaneously increased from 80 to 160 Hz. Since little change in the reflex bradycardia was elicited, increased impulse frequency at constant impulse number does not markedly increase reflex heart rate modification via the aortic nerve. This is supported by the finding that increases in the frequency of aortic nerve activity in the rabbit in response to augmented aortic blood pressure are the result of successive recruitment of nerve fibers having higher pressure thresholds rather than by increased firing rates of the individual fibers involved (2). The type of changes in nerve discharge that we produced by increasing discharge frequency at the expense of burst duration might, therefore, be expected to occur in response to increases in the slope and peak aortic pressure which are not accompanied by similar mean pressure changes. In the intact animal, such an increase in slope and peak of aortic pressure would stimulate a greater number of individual fibers within a shorter period of time leading to an increase in the whole-nerve discharge frequency during the systolic phase, although burst duration would be shorter for each cardiac cycle.

By contrast, when burst duration was fixed at 125 ms, doubling the impulse frequency from 80 to 160 Hz, which simultaneously increased total impulse number from 10 to 20, caused a 93% increase in peak reflex bradycardia (Fig. 1*B*). Previously, when impulse number was fixed (Fig. 1*A*), increased impulse frequency had little effect on the response. Consequently, impulse number in the aortic nerve appears to be an important reflex regulator of heart rate.

Finally, impulse frequency was held constant at 80 Hz while burst duration was increased from 62 to 125 ms which once again caused impulse number to double, this time from 5 to 10 (Fig. 1*C*). This caused a 70% increase in peak effect, again implicating the important influence of total pulse number. Previous studies have demonstrated that while individual baroreceptor fibers are recruited by increased systolic pressure in the aortic arch, increased mean arterial blood pressure leads to increased whole-nerve activity during the diastolic phase as well (1, 13). This causes an increase in impulse burst duration which may not be accompanied by changes in peak systolic discharge frequency. This condition is similar to that depicted in Fig. 1*C* which leads to augmented bradycardia.

DISCUSSION

The proximity of the aortic baroreceptors to the outflow of the left ventricle suggests that these receptors may play a vital role in the beat-to-beat regulation of the heart. Indeed, our results show that alterations in aortic nerve activity in a single cardiac cycle can elicit significant changes in the heart rate within the next heart cycle. The magnitude of the bradycardia is primarily dependent on the total number of impulses delivered within the stimulus burst. The offsetting influence of impulse frequency and burst duration on peak bradycardia, when impulse number was held constant, infers an approximately equal influence of frequency and duration. The nature of their inverse relationship, however, precludes determination of their independent influences on the reflex. Both the latency to onset and latency to peak effect, when evaluated in terms of the number of heartbeats following the stimulus burst, are independent of stimulus impulse number and frequency. Of course, the latency to peak effect, when evaluated as a function of time, increases as the response increases since the time between beats becomes greater. Increases in pulsatile aortic pressure have been shown to elicit increases in the frequency of aortic nerve activity via fiber recruitment primarily during the systolic phase (2). However, our results suggest that increased frequency of aortic nerve activity in itself does not increase the reflex heart rate

response when the stimulus burst duration is decreased. Indeed, augmented pulse pressure, which might lead to increased peak systolic discharge frequency and a shorter burst duration, has been shown to have small reflex cardiovascular effects (1, 14). However, elevation of mean arterial pressure increases the frequency of nerve activity during both systole and diastole (4, 13), thus increasing both stimulus burst duration and impulse frequency. Elevated mean arterial blood pressure has been shown to elicit potent reflex cardiovascular effects (1, 8).

Recent studies in which the carotid sinus nerve was stimulated indicate results similar to our findings for aortic nerve stimulation. In working unanesthetized dogs, Jonzon et al. (9) showed that continuous stimulation frequencies of 60–100 Hz caused a maximal initial effect on heart rate. This supports our earlier findings in the rabbit (11) as well as the results of others using anesthetized dogs (7). By contrast, steady-state blood pressure was maximally effected at stimulus frequencies in the 30- to 50-Hz range. In an allied study, Jonzon et al. (10) showed that the reflex blood pressure changes elicited by carotid sinus nerve stimulation (CSNS) were primarily dependent on impulse number rather than frequency. This supports our present findings using the aortic nerve. They showed that intermittent CSNS could elicit the same blood pressure effect as continuous CSNS at different frequencies. Their conclusions were that "central integrative functions" somehow reorder this pulsatile information and emit the appropriate efferent response without regard to afferent frequency per se. A similar mechanism may be operative in the aortic nerve control of heart rate.

Analysis of the rapid reflex control of heart rate via aortic baroreceptors on the basis of peak nerve discharge frequencies or total impulse numbers alone may not yield an accurate picture of how the entire reflex system responds to changes in arterial blood pressure. The discontinuous nature of baroreceptor nerve activity demands that more than impulse frequency and impulse number be studied. The slight increase in reflex bradycardia elicited by longer burst duration in the face of decreased impulse frequency indicates that the central integrative mechanism in concert with its efferent pathways may be sensitive to the total time of afferent nerve activity as well as to changes in impulse number.

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REFERENCES

1. ANGELL JAMES, J. E., AND M. DE B. DALY. Comparison of the reflex vasomotor responses to separate and combined stimulation of the carotid sinus and aortic arch baroreceptors by pulsatile and non-pulsatile pressures in the dog. *J. Physiol., London* 209: 257–293, 1970.
2. ANGELL JAMES, J. E. The effects of altering mean pressure, pulse pressure and pulse frequency on the impulse activity in baroreceptor fibers from the aortic arch and right subclavian artery in the rabbit. *J. Physiol., London* 214: 65–88, 1971.
3. ANGELL JAMES, J. E. Characteristics of single aortic and right subclavian baroreceptor fiber activity in rabbits with chronic renal hypertension. *Circulation Res.* 32: 149–161, 1973.
4. BERNE, R. M., AND M. N. LEVY. *Cardiovascular Physiology*. St. Louis: Mosby, 1972, p. 133.
5. CHALMERS, J. P., P. I. KORNER, AND S. W. WHITE. The relative roles of the aortic and carotid sinus nerves in the rabbit in the control of respiration and circulation during arterial hypoxia and hypercapnia. *J. Physiol., London* 188: 435–450, 1967.
6. DOUGLAS, W. W., AND W. SCHAUMANN. A study of the depressor and pressor components of the cat's carotid sinus and aortic nerves using electrical stimuli of different intensities and frequencies. *J. Physiol., London* 132: 173–186, 1956.
7. EDIS, A. J., AND J. T. SHEPHERD. Selective denervation of aortic arch baroreceptors and chemoreceptors in dog. *J. Appl. Physiol.* 30: 294–96, 1971.
8. GLICK, G., AND J. W. COVELL. Relative importance of the carotid and aortic baroreceptors in the reflex control of heart rate. *Am. J. Physiol.* 214: 955–961, 1968.
9. JONZON, A., P. A. OBERG, G. SEDIN, AND U. SJOSTRAND. Studies of blood-pressure regulation in the unanesthetized dog. I. The effects of constant-frequency stimulation of the carotid-sinus nerves. *Arch. Ges. Physiol.* 340: 211–228, 1973.
10. JONZON, A., P. A. OBERG, G. SEDIN, AND U. SJOSTRAND. Studies of blood-pressure regulation in the unanesthetized dog. II. The effects of impulse train stimulation of the carotid-sinus nerves. *Arch. Ges. Physiol.* 340: 229–249, 1973.
11. KARDON, M. B., D. F. PETERSON, AND V. S. BISHOP. Reflex bradycardia due to aortic nerve stimulation in the rabbit. *Am. J. Physiol.* 225: 7–11, 1973.
12. NEIL, E., C. R. M. REDWOOD, AND A. SCHWEITZER. Effects of electrical stimulation of the aortic nerve on blood pressure and respiration in cats and rabbits under chloralose and nembutal anesthesia. *J. Physiol., London* 109: 392–401, 1949.
13. PELLETIER, C. L., D. L. CLEMENT, AND J. T. SHEPHERD. Comparison of afferent activity of canine aortic and sinus nerves. *Circulation Res.* 31: 557–568, 1972.
14. SCHMIDT, R. M., M. KUMADA, AND K. SAGAWA. Cardiovascular responses to various pulsatile pressures in the carotid sinus. *Am. J. Physiol.* 223: 1–7, 1972.

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Left ventricular function during acute regional myocardial ischemia in the conscious dog

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BISHOP, VERNON S., ROBERT L. KASPAR, GEORGE E. BARNES, AND MERRILL B. KARDON. *Left ventricular function during acute regional myocardial ischemia in the conscious dog*. J. Appl. Physiol. 37(6): 785–792. 1974.—The effects of acute 1-min occlusion of the left circumflex coronary artery on the inotropic state and performance of the left ventricle were examined in adult mongrel dogs. The inotropic state, as indicated by changes in the maximum derivative of left ventricular pressure in the preejection phase and the maximum derivative of the transverse internal diameter, were diminished during the ischemic period. The end-systolic diameter increased 3.8 ± 0.6 mm while the end-diastolic diameter increased only 0.4 ± 0.2 mm, although the end-diastolic pressure increased 6.9 ± 0.6 mmHg. Progressive decreases in the stroke volume paralleled the apparent reduction in myocardial fiber shortening in the transverse plane. Cardiac output and arterial pressure declined, concurrently, thus maintaining a constant peripheral resistance during the occlusion. Acute coronary occlusion also caused an apparent increase in the myocardial wall stiffness as judged by the increase in the slope a of the equation $dP/dV = aP + B$, the increase of the slope of the pressure-diameter relationship, and the decrease in the rate of lengthening of the diameter during diastole. These findings suggest that acute myocardial ischemia results in an immediate reduction in the effective inotropic state and an apparent increase in the myocardial wall stiffness in the transverse plane. Both of these changes resulted in a decrease in performance of the left ventricle.

coronary artery occlusion; myocardial fiber shortening; dP/dt

ALTHOUGH NUMEROUS STUDIES have demonstrated that left ventricular function is compromised during myocardial ischemia (7, 9, 11, 12) and infarction (16, 17), relatively little is known concerning the immediate changes in left ventricular function in conscious animals during acute myocardial ischemia. Most of our present information has been derived either from acute animal studies (7, 12, 31) or from patients during stress-induced angina (11). Furthermore, recent studies in patients have suggested that, during ischemia, increases in myocardial wall stiffness may severely limit and cause misinterpretation of the Frank-Starling reserve (9, 11). Therefore it is the purpose of this study to evaluate, in conscious dogs, the reduction in left ventricular performance during acute regional myocardial ischemia. We attempted to identify, as contributing factors, changes in the inotropic state, extent of myocardial fiber shortening, and ventricular wall stiffness.

To accomplish these aims, left ventricular pressure, transverse internal diameter, and stroke volume were measured before and during acute 1-min occlusions of the left circumflex coronary artery.

METHODS

Surgery. In 13 adult mongrel dogs (16–20 kg) sterile thoracotomies were performed under methoxyflurane anesthesia. By using the technique previously described (3, 4, 20), two sonomicrometer transducers were implanted on the endocardial surface of the left ventricle—one on the anterior and the other on the posterior left ventricular wall. Through a second stab wound near the apex a calibrated solid-state pressure transducer (Konigsberg Instruments, P-18) was implanted in the left ventricle. A previously calibrated electromagnetic flow probe was placed around the ascending aorta, and an 18-gauge polyvinyl catheter was placed in the left atrial appendage. The left circumflex coronary artery was exposed close to its origin and an occlusive device, similar to that reported by Chimoskey et al. (8), was placed proximal to the first segment of the vessel. In some animals a small electromagnetic flow probe was placed proximal to the cuff. A careful dissection was performed in order to minimize any gross damage to the nerve supply of the artery. Occasionally, small branches of the *left circumflex* were sacrificed during the dissection, but in no circumstances did this lead to a damaged area as judged from visual observations and pathological examination at autopsy. The electrical leads, catheters, and distal end of the occluding device were exteriorized at the back of the neck. Approximately two weeks were allowed for recovery and, at the time coronary occlusions were performed, body temperature and ECG were normal.

Measuring of flow, pressure, and diameter. A Zepeda SW F1 electromagnetic flowmeter was used to detect aortic flow and coronary blood flow. The flow probes were calibrated in vitro before implantation and rechecked after the animals were killed. In some cases the in vivo calibration of the aortic flow probes was checked by using dye dilution techniques, and in all cases the calibrations agreed within 5%. The signal in late diastole was assumed to represent zero aortic flow. Zero coronary flow was determined by occlusion of the coronary artery. Stroke volume was obtained from aortic flow by analog integration of each ejection period, using a Philbrick operational amplifier.

Left ventricular transverse internal diameter was obtained by use of a sonomicrometer which measures the mean transit time for a burst of 5 mHz ultrasound between the two piezoelectric crystals at a sampling rate of 5,000 times per second (30). Since the velocity of sound in blood is known, transit time is convertible to distance.

Left ventricular pressures were measured by Konigsberg Instrument P-18 solid-state pressure transducers (3, 18–20). The sensitivity of the transducers normally remains stable during implantation. On occasion, under local anesthetics, catheters were inserted into the left ventricle to verify the calibrations of the transducers. The reliability of the transducers was also estimated by comparing the left ventricular pressure with the arterial pressure at the time of ejection. Since a routine check of zero left ventricular pressure by a catheterization technique was not possible, the zero drift was normally corrected by adjusting left ventricular end-diastolic pressure to equal mean left atrial pressure at the beginning of the experiment while the animal was resting quietly on its right side (3, 4, 28).

Differentials of pressure and diameter were obtained by using an SQ10A operational amplifier (Analog Devices) or by digital techniques (25). The analog derivative was checked by differentiating a sine wave, using the operational amplifier. The phase shift was 90° and the amplitude of the differential output was linear between 0.5 and 100 Hz. The differential was calibrated using a triangular wave.

Mean arterial pressures were recorded through a catheter implanted in the left carotid artery. Routine electrocardiograms were recorded with subcutaneous needle electrodes. Electrical evaluation of the ischemia produced during coronary occlusion was obtained with a standard precordial electrocardiogram, with the indifferent electrode connected to the ground of the coronary flow probe when the probe was not used. Peaking of the T-wave with S-T segment elevation was observed in each animal during the period of ischemia.

Recording. All signals were inscribed simultaneously on a Type R Beckman oscillographic recorder and an Ampex FR 1300 magnetic tape recorder. Tapes were analyzed with a Philco 3000 Digital computer after analog-to-digital conversion. The left ventricular internal diameter and aortic flow and the integral of aortic flow were examined as a function of the R-R interval of the electrocardiogram. Integrated aortic flow and left ventricular pressure were computed as a function of left ventricular diameter (3, 4, 18, 28).

Protocol. The coronary artery occlusive devices were checked in vitro as to the pressure and volume required to occlude the various size arteries. To assure the effectiveness of the occluding device, in vivo transient occlusions were performed at the time of implantation. During the occlusion experiments identical volumes and pressures were used to occlude the coronary artery. In those animals with coronary flow probes the occlusion of the circumflex coronary artery eliminated blood flow into the artery within 3 s.

Resting measurements were obtained while the animal was lying quietly on its right side, unsedated and unrestrained. Following these measurements the left circumflex coronary artery was occluded for 1 min and released. Additional occlusions were performed only after all parameters had returned to the preocclusive level. This was accomplished within 90 s. Occlusions were performed on at least two separate days in each animal, and hemodynamic alterations were reproducible at ± 10%. As demonstrated by the statistical analysis in Table 1, the group response was also reproducible. Statistical comparisons were made using the t test for paired data. P values < 0.05 were considered significant.

To evaluate the effects of increases in the initial preload, the left ventricular end-diastolic pressure (LVEDP) and the end-diastolic diameter (EDD) were increased in five animals by intravenous infusion of Tyrode solution (37°C) (2, 3) and occlusion was performed. The LVEDP was raised in each animal to a level significantly higher than the control level, but care was taken not to raise the LVEDP to levels which produce reflex tachycardia.

In three animals, changes in EDD during elevation in preload by intravenous infusions of Tyrode solution were compared to the changes in EDD resulting from coronary occlusion. To maintain a continuous rise in LVEDP, similar to that observed during acute coronary occlusion, Tyrode solution was infused (300–400 ml/min) into a catheter in the left jugular vein. The total volume infused ranged from 400 to 600 ml. This volume did not significantly alter the hematocrit (3, 4). The increments in EDD resulting from these increases in LVEDP were compared to those observed during coronary artery occlusion at similar LVEDP.

In four additional animals instrumented with aortic flow probes, infusions were performed using the above technique. When the stroke volume became fixed at a maximum level, mean left atrial pressure response was noted.

Estimation of ventricular wall stiffness. The passive elastic modulus during diastole, as defined by Diamond and Forrester and associates (9, 10) was used to estimate the change in myocardial wall stiffness during coronary artery occlusion. With this method the equation $dP/dV = aP + B$ linearly relates the reciprocal of compliance during diastole to the pressure during diastole. The slope a of the equation is used to signify changes in myocardial wall stiffness. The derivative of pressure (dP) is approximated by the change in left ventricular pressure during diastole and is equal to the left ventricular end-diastolic pressure (LVEDP) minus the left ventricular pressure following systole, ESP (ΔP = EDP − ESP, Fig. 1). The derivative of volume (dV) was

TABLE 1. *Average values and mean changes during coronary artery occlusion in 13 dogs*

Condition	Mean Cardiac Output, (ml/kg·min)	Stroke Volume, (ml/beat·kg)	Heart Rate, beats/min	Arterial Pressure, mmHg
Control	171±27	1.49±0.29	114±12	101±9
Coronary occlusion	144±31	1.00±0.23	148±12	85±18
Mean difference	−27±6†	−0.50±0.30†	35±9†	−16±6*
No. of paired observations	13	13	13	9

Values corresponding to control and during coronary occlusino are means ± SEM. Mean difference ± SD compares the changes between control and coronary occlusion. * $P < 0.05$, † $P < 0.001$.

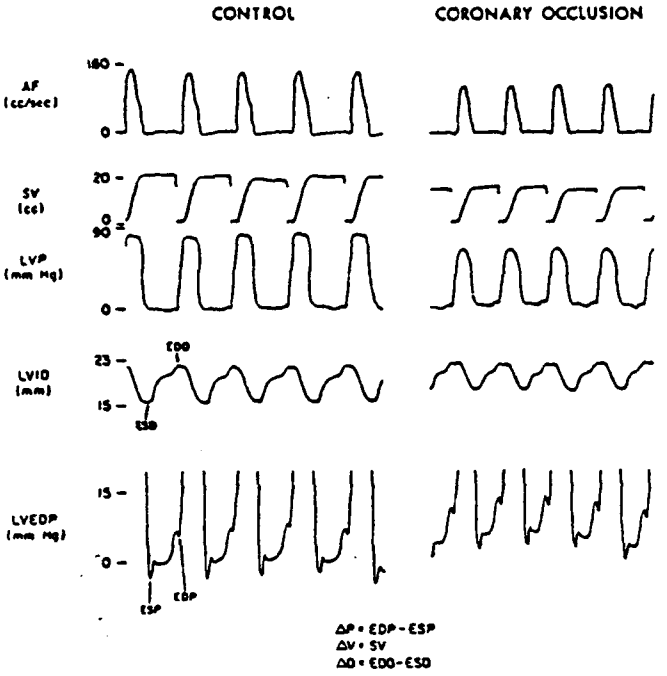

FIG. 1. Analog recording of aortic flow (AF), stroke volume (SV), left ventricular pressure (LVP), left ventricular internal diameter (LVID), and left ventricular end-diastolic pressure (LVEDP) is shown before and during the peak response to occlusion of the left circumflex coronary artery. ESP = minimum left ventricular pressure after systole; EDP = LVEDP; EDD = left ventricular internal end-diastolic diameter; and ESD = left ventricular internal end-systolic diameter. The change in volume (ΔV) during diastole is equated to the preceeding stroke volume (SV).

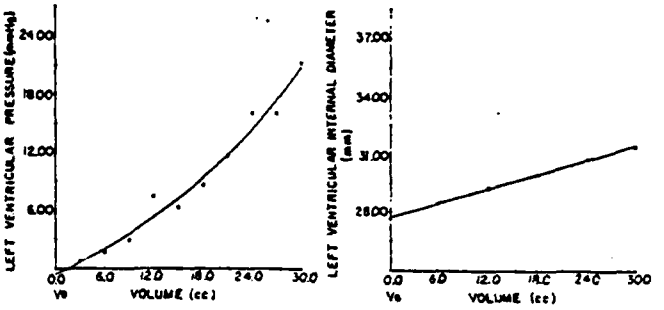

FIG. 2. Relationship of left ventricular internal diameter and pressure to volume in a nonbeating heart.

approximated by the stroke volume during a steady-state condition. In the present study, the slope a of the above relationship was evaluated in five dogs before and during occlusion of the coronary artery. Calculations of dP and P were similar to those described by Diamond and Forrester and coworkers (9, 10).

Critique of methods. Investigators using a variety of techniques have confirmed that the canine left ventricle ejects blood primarily by shortening in the transverse plane and that changes in the apex-base plane are slight (1, 29). Studies in this laboratory have established a linear relationship between the volume ejected and the greatest transverse internal diameter (3, 4, 18, 28). Left ventricular internal diameter and volume changes have been related in nonbeating hearts as well as in the spontaneously beating hearts of conscious dogs (2, 6). Furthermore, during passive distension of the left ventricle in nonbeating hearts, diameter changes linearly with volume, while pressure varies as an exponential function of volume as shown in Fig. 2. In six animals the linearity of the diameter-volume relationship was highly significant ($P < 0.001$), as was the exponential relationship of pressure and volume. Thus, these experiments confirm the utility and reliability of the use of left ventricular transverse internal diameter as an index of volume change.

The placement of the sonomicrometer transducers with respect to the ischemic tissue was evaluated by: *1*) injecting latex or silastic into the left circumflex coronary artery at autopsy, and dissecting the area of distribution (7 dogs); *2*) electrocardiographic changes in one acute dog, using electrodes placed directly on the left ventricle; *3*) visual observations of discoloration during a test occlusion at the time of surgery (all dogs); and *4*) histological sections following a permanent occlusion (1 dog). In all cases the transducers were implanted in normal myocardial tissue. In four additional dogs myocardial blood flow distribution was evaluated before and during coronary occlusion by using the radioactive microsphere technique. The perfusion of the ventricular tissue surrounding the area of implantation was unaltered by coronary occlusion.

Myocardial wall stiffness. Recent studies have provided a clearer insight into the pressure-volume (P-V) relationship of the left ventricle (9, 10). The P-V relationship obtained in these studies was approximated by an exponential relationship. The reciprocal of compliance, dP/dV, was thus a linear function of pressure (P). The slope a of the equation $dP/dV = aP + B$ was shown to be related to the wall stiffness and was termed the passive elastic modulus. This index was a quantitative measure of left ventricular wall stiffness; the wall stiffness is a measure of the passive stress-strain characteristic of the left ventricle and determines how well the ventricle will expand for a given diastolic pressure. The slope a was independent of ventricular size and rate of change of pressure, and was only slightly affected by changes in left ventricular geometry. $\Delta P/\Delta V$ is an average of dP/dV during diastole. Since the pressure gradients from the beginning to end of diastole are relatively small, further division of the gradients would result in additional inaccuracies. Another approach would be to take the differential of the left ventricular pressure and diameter during diastole, but, reliable differentials of the pressure during this portion of the cardiac cycle are difficult to obtain. Thus, in our conscious animal studies, the $\Delta P/\Delta V$ obtained by the method of Diamond appears preferable to other methods.

RESULTS

Thirty-eight acute coronary occlusions were performed in 13 conscious dogs. The average response and the mean differences are shown in Table 1 and Table 2. In eleven animals, 1-min occlusions of the left circumflex coronary artery did not result in an overt display of pain. Two animals may have had a moment of discomfort, for each extended its left leg at the onset of the occlusion period.

Figure 3 illustrates a typical ventricular response to acute

TABLE 2. *Average values and mean changes during coronary artery occlusion in 13 dogs*

Condition	Left Ventricular Pressure (LVP)		Left Ventricular Internal Diameter (LVID)		Peak dD/dt		Peak dP/dt	
	Peak systolic, mmHg	End diastolic, mmHg	End diastolic, mm	End systolic, mm	Systole, mm/s	Diastole, mm/s	Pre-ejection, mm/s	Relaxation, mm-Hg/s
Control	115 ±23	6.2 ±1.7	31.3 ±8.2	23.2 ±7.8	−67 ±14	99 ±18	2813 ±663	2398 ±588
Coronary occlusion	101 ±22	13.3 ±2.5	31.6 ±8.1	27.0 ±8.2	−49 ±10	59 ±17	2269 ±463	1788 ±534
Mean difference	−15 ±3*	6.9 ±0.7†	0.4 ±0.2*	3.8 ±0.6†	−18 ±4†	−41 ±4†	−542 ±103†	−623 ±84

Values corresponding to control and during coronary occlusion are mean ± SEM. Mean difference ± SD compares to changes between control and coronary occlusion. dD/dt, Maximum derivative of LVID. dP/dt, Maximum derivative of LVP. * $P < 0.05$, † $P < 0.01$.

occlusion of the left circumflex coronary artery. As coronary blood flow fell to zero, left ventricular end-systolic diameter (ESD), heart rate (HR), and left ventricular end-diastolic pressure (LVEDP) increased progressively toward a level which was constant during the occlusive period. Maximum changes were reached 37.8 ± 8.5 s after onset of the occlusion. This response was reproducible in a single animal or group of animals from day to day. During the 1-min occlusion there was peaking of the T-wave on the electrocardiogram. Ventricular ectopic beats or premature beats were seldom seen during occlusive periods. Postocclusion recovery of left ventricular performance was complete within 90 s.

The most dramatic response to occlusion of the coronary artery was observed in the elevation of the end-systolic diameter (3.8 ± 0.6 mm) (Fig. 3, Table 2). The average latency for the initial changes in ESD was 5.6 ± 1.9 s. In contrast, the end-diastolic diameter (EDD) was little affected by the occlusion despite increases in filling pressure (LVEDP, 6.9 ± 0.7 mmHg). The decline in stroke volume (−0.50 ± 0.30 ml·beats·kg) (Table 1) reached a maximum value in 37.8 ± 8.5 s and was correlated with the reduction in the extent of shortening in the transverse plane. Heart rate was elevated in each animal in response to the occlusion of the left circumflex coronary artery (35 ± 9 beats·min; Table 1) (26) and the cardiac output fell progressively during the occlusions, reaching a maximum decrease (−27 ± 6 ml/min·kg; Table 1) at 27.7 ± 7.8 s. The cardiac output was maintained in some animals when the elevation in heart rate offset the fall in stroke volume. When the cardiac output was maintained by the tachycardia, the extent of the fall in arterial pressure was reduced and the onset of the change delayed.

Left ventricular peak systolic pressure decreased an average of 15 ± 3 mmHg. The maximum derivatives of left ventricular pressure during systole (dP/dt) and during relaxation (dP/dt) decreased 542 ± 103 mmHg/s and 623 ± 84 mmHg/s, respectively (Table 2). The peak shortening rate, as indicated by the derivative of the left ventricular internal diameter during ejection, (−dD/dt)

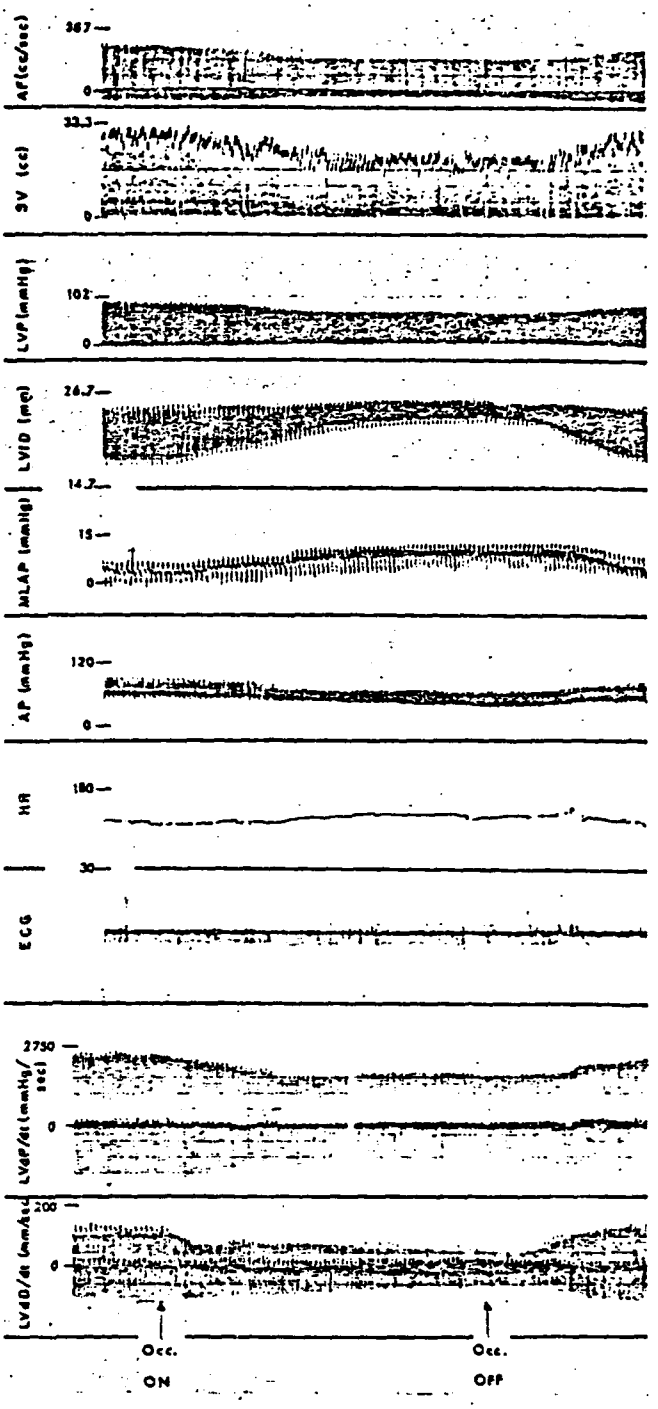

FIG. 3. An analog recording as obtained before occlusion (Occ. ON) of the left circumflex coronary artery, during occlusion and immediately following the occlusion (Occ. OFF). The recordings from the *top* to *bottom* are aortic blood flow (AF), stroke volume (SV), left ventricular pressure (LVP), left ventricular transverse internal diameter (LVID), mean left atrial pressure (MLAP), arterial pressure (AP), heart rate (HR), electrocardiogram (ECG), derivative of left ventricular pressure (LV dP/dt), derivative of left ventricular internal diameter (LV dD/dt). Time between occlusion on (Occ. ON) and occlusion off (Occ. OFF) is 1 min.

fell from 67 ± 14 mm/s to 49 ± 10 mm/s while the peak rate of lengthening $(dD^r)/dt$ of the diameter declined from 99 ± 18 mm/s to 59 ± 17 mm/s (Table 2). The latencies for alterations in the derivatives of pressure and diameter were similar and occurred immediately following the increase in ESD.

Figure 4A illustrates aortic flow, left ventricular internal diameter, and pressure as a function of time between the R-R interval before and during the peak response to coronary occlusion. The normal near-linear relationship between left ventricular internal diameter and stroke volume was maintained throughout the ischemic period. The reduction in volume ejected was the result of an increase in end-systolic diameter since the slope of the stroke volume-diameter relationship was not significantly altered (Fig. 4*B*). Fig. 5 shows the average changes that occur in stroke volume and left ventricular internal diameter during occlusion. The change in stroke volume was directly related to the reduction in stroke diameter (EDD-ESD) during the occlusion (Fig. 5*B*).

In Fig. 6, EDD and ESD are plotted as functions of LVEDP in a single animal. When left ventricular end-diastolic pressure was increased by intravenous infusion of Tyrode solution, the increment in EDD exceeded the increment in ESD. On the other hand, when the LVEDP was increased in response to the reduction in coronary blood flow, there was little change in EDD, while ESD increased beyond that observed during the intravenous infusion. In both of the conditions illustrated the heart rate response was similar. Similar differences in the response to increments in LVEDP resulting from intravenous infusion and

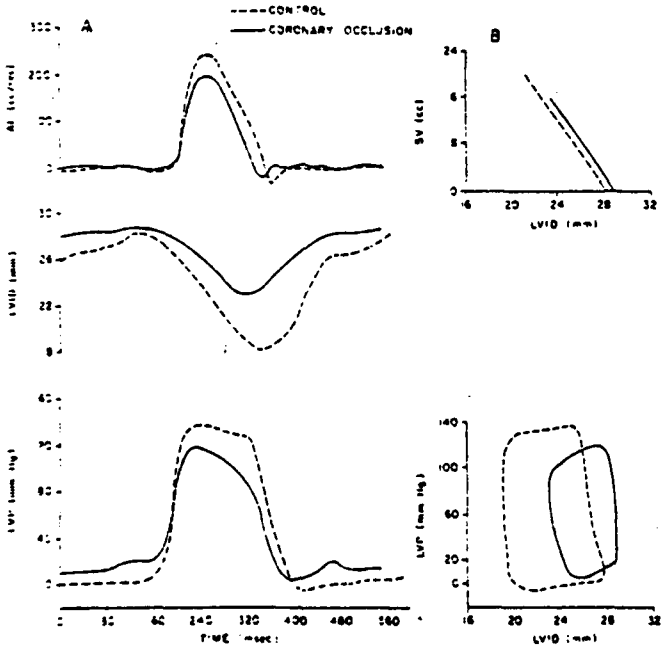

FIG. 4. *A:* Digital computer printout of aortic flow (AF), left ventricular internal diameter (LVID), and left ventricular pressure before and during the peak response to coronary occlusion. *B:* Relationship of stroke volume (SV) and left ventricular pressure to left ventricular internal diameter (LVID). Each curve is an average of 10 consecutive beats.

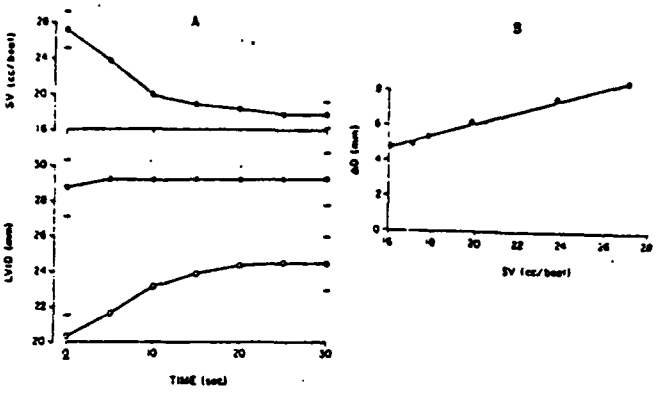

FIG. 5. *A:* The effects of coronary occlusion on stroke volume (SV) and left ventricular internal diameter (LVID) in 7 dogs. ● = left ventricular internal end-diastolic diameter (EDD); ○ = left ventricular internal end-systolic diameter (ESD). *B:* Relationship between stroke volume and stroke diameter (ΔD) during occlusion. ΔD = EDD − ESD.

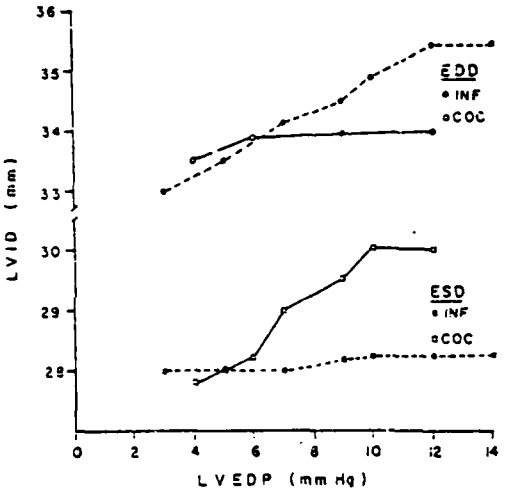

FIG. 6. Comparison of changes in left ventricular internal diameter during diastole (EDD) and systole (ESD) during elevations in filling pressure resulting from isotonic intravenous infusion of Tyrode's solution (INF) or due to acute occlusion of left circumflex coronary artery (COC).

coronary occlusion were observed in three animals studied. When coronary occlusion was performed following initial increases in LVEDP (7.0 mmHg) and EDD (1.1 mm), LVEDP was significantly elevated over the control response (14.0 ± 2.2 mmHg), while EDD was unaltered (Table 3). With this exception, the ventricular responses to coronary occlusion before and after increases in preload were similar.

Figure 7 illustrates changes which occur in stroke volume and mean left atrial pressure as a result of coronary occlusion at rest and at the peak of the intravenous infusion. At rest, coronary occlusion decreased the stroke volume 5.4 ml (17.2 − 11.8 ml) and increased the mean left atrial pressure 7.0 mmHg. Acute volume loading during control states increased the stroke volume to a maximum value of 22.8 ml with a change of 10 mmHg in mean left atrial pressure. Subsequent occlusions of the coronary artery

TABLE 3. *Mean changes to coronary occlusion before and after preload in five dogs*

Condition	Left Ventricular Pressure (LVP)	Left Ventricular Internal Diameter (LVID)		Peak dD/dt
	End diastolic, mmHg	End diastolic mm	End systolic, mm	Diastole, mm/s
Control occlusion	6.4±1.2†	0.5±0.1*	3.1±0.7*	−34±4†
Increased preload occlusion	14.0±2.2†	0.2±0.2	3.3±0.8†	−38±7†

Mean difference ± SD resulting from coronary artery occlusion are shown before (control occlusion) and following increased preload occlusion. In the latter, the initial increment in preload resulted in mean increases in left ventricular end-diastolic pressure (7.0 ± 1.0 mmHg) and end-diastolic diameter (1.1 ± 0.3 mm). dD/dt, Maximum derivative of LVID. * $P < 0.05$, † $P < 0.01$.

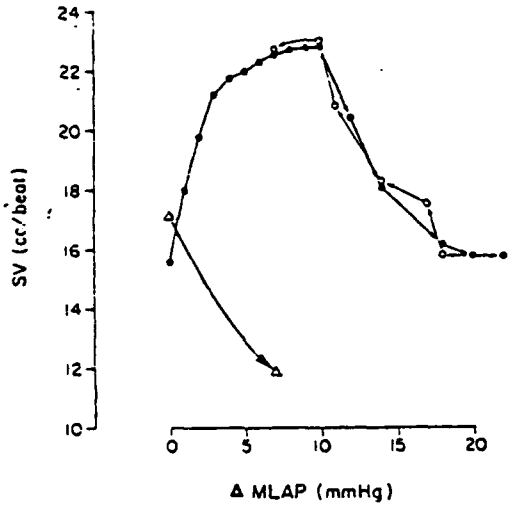

FIG. 7. Relationship of stroke volume and mean left atrial pressure in a single conscious animal: during coronary occlusion (Δ——Δ); during infusion (0–10 mmHg) (●——●) and during occlusion following infusion (10–22 mmHg) (●——●); and during recovery following release of the occlusion (20–7 mmHg) (○——○). At the peak of the stroke volume curve (SV = 22.8 cc and Δ MLAP = 10 mmHg) the left circumflex was occluded. The occlusion was released at a stroke volume of 15.8 cc and a Δ MLAP of 22 mmHg. The open circles (○——○) indicate the return of the parameters to preocclusion values.

reduced the stroke volume to 15.7 ml and increased the change in mean left atrial pressure to 22 mmHg. As shown in Fig. 7, release of the occlusion resulted in a return of the stroke volume to the original curve. Because of the large increases in mean left atrial pressure, stroke volume output curves were not attempted during occlusion.

In Fig. 8, $\Delta P/\Delta V$ is shown as a function of the mean left ventricular diastolic pressure ($\bar{P}$) during the peak response to coronary occlusion. A significant linear relationship was obtained between $\Delta P/\Delta V$ and mean left ventricular diastolic pressure during control states and during coronary occlusion. The slope of the curve provides an index of left ventricular wall stiffness, *a*. During occlusion, the slope *a* was significantly elevated ($P < 0.01$), suggesting that the wall stiffness increased during acute regional myocardial ischemia. The increase in wall stiffness observed following occlu-

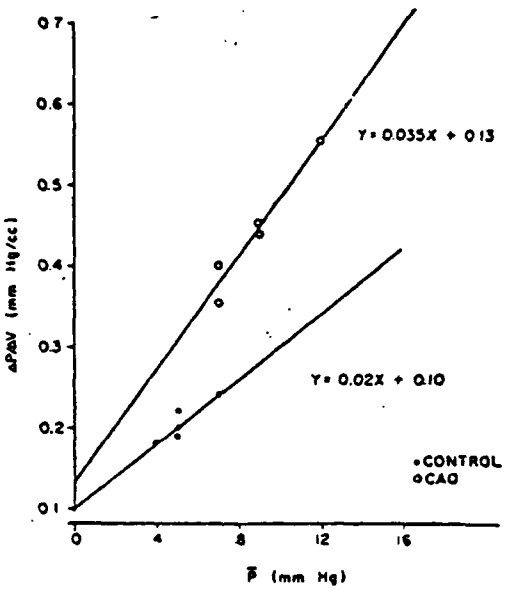

FIG. 8. $\Delta P/\Delta V$ is plotted versus mean diastolic pressure $\bar{P}$. The stroke volume was used as a measure of ΔV. The correlation coefficient for the control data (●) is $r = 0.891$, $P < 0.02$. For curve representing the peak effect during coronary occlusion (○) the correlation coefficient is $r = 0.970$, $P < 0.01$. The slopes were significantly different ($P < 0.01$).

sion of the left circumflex coronary artery is also illustrated by the alteration in the left ventricular diastolic pressure-diameter curves shown in Fig. 4*B*. During coronary occlusion the left ventricular end-diastolic diameter is not significantly increased, although the LVEDP is substantially elevated. For any given diastolic diameter, the left ventricular pressure is higher than that observed during the control state. This results in an increase in the slope of the pressure-diameter relationship during coronary artery occlusion. The slope of the pressure-diameter curve increased from 2.33 ± 0.41 to 4.58 ± 0.65 mmHg/mm during coronary artery occlusion.

As stated above, these observed changes in wall stiffness were accompanied by reductions in the inotropic state of the heart as reflected by significant reductions in dP/dt (max) and dD/dt (max). However, there was a much larger reduction in the derivative of the left ventricular internal diameter during relaxation (41%). This reduced rate of myocardial muscle fiber lengthening, coupled with an elevated left ventricular end-diastolic pressure, is consistent with the observed changes in myocardial wall stiffness.

DISCUSSION

This study provides the first comprehensive analysis of the effects of regional myocardial ischemia on the left ventricle of conscious dogs. The responses to repeated 1-min occlusions of the left circumflex coronary artery were reproducible with respect to time, to maximum change, and to the extent of the change. The maximal change occurred at 38 s after the onset of the occlusion, with no later evidence of changes in ventricular function. The diameter recordings before, during, and after occlusion were similar in contour.

Acute regional myocardial ischemia resulted in a reduction in the inotropic state of the heart as assessed by the observed decreases in dP/dt (max) and dD/dt (max). Both of these variables have been previously shown to be sensitive indices of the inotropic state (1, 14). Furthermore, it is unlikely that these changes were significantly influenced by the alterations which occurred in left ventricular end-diastolic pressure, heart rate, or arterial pressure, all of which have previously been shown to have minimal effects on either dP/dt (max) or dD/dt (max) (1). Reductions in dP/dt (max) and dD/dt (max) most likely resulted from the reduced contractile state of the ischemic portion of the myocardium. The extent of shortening from any given initial length was also severely compromised during the ischemic period and resulted in a proportional reduction in the stroke volume (Fig. 5). Since the relationship between diameter and stroke volume is unaltered during ischemia, one must assume the left ventricle is still ejecting blood primarily by shortening in the transverse plane (9). The degree of systolic ballooning (7, 16, 27, 32, 33) or asynchronous contraction could not be evaluated in the present study. The fact that the contour of the diameter recording was similar before and during the occlusion suggests that the effects of asynchrony were small. Placement of the transducers in normal tissue may have minimized the effect. It also seems unlikely that systolic ballooning, a condition which disassociates the changes in stroke volume from the extent of shortening, could account for the above observations. A more likely explanation would be the loss of functional myocardial tissue. The ventricle is essentially a series arrangement of myocardial muscle fibers, and for this reason, changes in the mechanics of the ischemic area can affect the entire ventricle (15, 21). Therefore, the reduction in dP/dt (max), dD/dt (max), and the extent of shortening may be due to a loss of functional myocardial tissue (hypokinesis) in the ischemic tissue. In isolated heart studies, similar reductions in the extent and rate of shortening and the rate of tension developed have been observed during hypoxia (34, 35).

It is apparent from our results that regional myocardial ischemia alters the normal relationship between left ventricular filling pressure and the end-diastolic diameter. During ischemia, LVEDP increased without a corresponding change in EDD. The slope of the pressure-diameter relationship was also elevated. Assuming that changes in left ventricular end-diastolic diameter reflect changes in end-diastolic volume, it appears likely that the myocardial wall is less distensible in the transverse plane. The above assumption is supported by the following evidence. *1*) The left ventricle is part of a closed fluid-filled system and, as such, changes in diameter during systole and diastole most likely represent the same volume. *2*) Mitchell et al. (25) have shown that ventricular volume changes during systole and diastole are reflected by identical changes in the cross-sectional area of the left ventricle. *3*) In the nonbeating heart, we have shown that left ventricular volume and diameter change together while pressure and volume are exponentially related (Fig. 1).

Using a more direct approach for estimating myocardial wall stiffness, we observed dramatic changes in stiffness during ischemia. The index of stiffness during ischemia was similar to that previously reported in patients with coronary artery disease (9), but different from that reported in open-chested animals during acute infarction (13). However, it is likely that in these studies the initial state of the myocardium was compromised by the anesthetic and experimental procedures. In our study the increment in stiffness begins to take place shortly after the occlusion, and a stable maximum increase in myocardial wall stiffness is reached 30–60 s into the occlusion. When the occlusion is released, the stiffness begins to return to its control value, and it is fully restored to its control value 15–30 s after the release. The rapid increases and restorations of myocardial stiffness indicated that, at least acutely, these apparent changes in myocardial stiffness are related to the direct effect of ischemia on the myocardium. Because of the rapidity and reversibility of the changes, it is likely that alterations are occurring in the biochemical mechanism responsible for relaxation (22–24).

Myocardial ischemia reduces the performance of the heart, as illustrated by the reduction in the stroke volume at rest and at the peak of the infusion response. Because the extent of shortening from any given initial muscle length is severely compromised, this reduction in the Frank-Starling reserve is obviously related in part to the decline in the inotropic state. Although not so obvious, increases in myocardial wall stiffness may also have important functional significance, since the Frank-Starling reserve of the heart is dependent on the pressure-volume characteristics as well as the ability of the heart to develop force in response to distension. When the myocardial wall stiffness is elevated, greater increments in left ventricular filling pressures are required to stretch the myocardium. Consequently, although the myocardium may still be capable of developing an increased force, the stroke volume or stroke work response is less for any given increase in filling pressure. Thus, increments in myocardial wall stiffness may cause misinterpretation of the classic Frank-Starling function curve; but, more importantly, it limits the Frank-Starling reserve of the heart, for extremely high filling pressure may be required to stretch the myocardium.

Several investigators (5, 9) have questioned, particularly in the diseased heart, the reliability of left ventricular end-diastolic pressure as an index of changes in left ventricular end-diastolic size. Increases in left ventricular stiffness or a failing heart could both result in similar changes in left ventricular end-diastolic pressure. As pointed out by Diamond and Forrester (9), a ventricular function curve may not express the difference between changes in ventricular stiffness and reduced inotropic state. Thus, without an index of ventricular end-diastolic volume, the exact functional status of the myocardium may be impossible to determine. In the present study, failure to increase the stroke volume during increments in LVEDP could be interpreted as simply a reduction in ventricular function; however, the limitation of ventricular function may be in part due to the apparent increase in stiffness of the left ventricle.

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REFERENCES

1. Barnes, G., V. S. Bishop, L. D. Horwitz, and R. L. Kaspar. The maximum derivatives of left ventricular pressure and transverse internal diameter as indices of the inotropic state of the left ventricle in conscious dogs. *J. Physiol., London.* 235: 571–590, 1973.
2. Bishop, V. S., and L. D. Horwitz. Left ventricular transverse internal diameter: Value in studying left ventricular function. *Am. Heart J.* 80: 507–514, 1970.
3. Bishop, V. S., and L. D. Horwitz. Effects of altered autonomic control on left ventricular function in conscious dogs. *Am. J. Physiol.* 221: 1278–1282, 1971.
4. Bishop, V. S., L. D. Horwitz, H. L. Stone, H. F. Stegall, and E. J. Engelken. Left ventricular internal diameter and cardiac function in conscious dogs. *J. Appl. Physiol.* 27: 619–623, 1969.
5. Braunwald, E., and J. Ross, Jr. Ventricular end-diastolic pressure: apprisal of its value in the recognition of ventricular failure in man. *Am. J. Med.* 34: 147–150, 1963.
6. Carleton, R. A., and J. G. Clark. Measurement of left ventricular diameter in the dog by cardiac catheterization. *Circulation Res.* 22: 545–558, 1968.
7. Case, R. B., E. Berglund, and S. J. Sarnoff. Ventricular function. II. Quantitative relationship between coronary flow and ventricular function with observations on unilateral failure. *Circulation Res.* 2: 319–325, 1954.
8. Chimoskey, J. E., M. Szentivanyi, R. Zakheim, and A. C. Barger. Temporary coronary occlusion in conscious dogs: collateral flow and electrocardiogram. *Am. J. Physiol.* 212: 1025–1032, 1967.
9. Diamond, G., and J. S. Forrester. Effect of coronary artery disease and acute myocardial infarction on left ventricular compliance in man. *Circulation.* 45: 11–19, 1972.
10. Diamond, G., J. S. Forrester, J. Hargis, W. W. Parmley, R. Danzig, and H. J. C. Swan. Diastolic pressure-volume relationship in the canine left ventricle. *Circulation Res.* 29: 267–275, 1971.
11. Dwyer, E. W. Left ventricular pressure-volume alteration and regional disorders of contractions during ischemia induced by atrial pacing. *Circulation.* 42: 1111–1122, 1970.
12. Enright, L. P., H. Hannah, and R. L. Reis. Effects of acute regional myocardial ischemia on left ventricular function in dogs. *Circulation Res.* 26: 307–315, 1970.
13. Forrester, J. S., G. Diamond, W. W. Parmley, and H. J. C. Swan. Early increases in left ventricular compliance after myocardial infarction. *J. Clin. Invest.* 51: 598–603, 1972.
14. Furnival, C. M., R. J. Linden, and H. M. Snow. Inotropic changes in the left ventricle: The effect of changes in heart rate, aortic pressure and end-diastolic pressure. *J. Physiol., London.* 211: 359–387, 1970.
15. Herman, M. V., and R. Gorlin. Implications of left ventricular asynergy. *Am. J. Cardiol.* 23: 538–547, 1969.
16. Hood, W. B., Jr., V. H. Covelli, W. H. Abelmann, and J. C. Norman. Persistance of contractile behavior in acutely ischemic myocardium. *Cardiovascular Res.* 3: 249–260, 1969.
17. Hood, W. B., Jr., J. Joison, R. Kumar, I. Katayama, R. S. Neiman, and J. C. Norman. Experimental myocardial infarction. I. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. *Cardiovascular Res.* 4: 73–83, 1970.
18. Horwitz, L. D., and V. S. Bishop. Left ventricular pressure-dimension relationships in the conscious dog. *Cardiovascular Res.* 6: 163–171, 1972.
19. Horwitz, L. D., and V. S. Bishop. The effect of acute volume loading on heart rate in the conscious dog. *Circulation Res.* 30: 316–321, 1972.
20. Horwitz, L. D., V. S. Bishop, H. L. Stone, and H. F. Stegall. Continuous measurement of internal left ventricular diameter. *J. Appl. Physiol.* 24: 738–740, 1968.
21. Jóhannsson, M., and E. Nilsson. A note on the influence of asynchronous activation of myocardial contraction. *Acta Physiol. Scand.* 85: 242–248, 1972.
22. Katz, A. M., and D. I. Repke. Quantitative aspects of dog cardiac microsomal calcium binding and calcium uptake. *Circulation Res.* 21: 153–162, 1967.
23. Katz, A. M., D. I. Repke, and B. R. Cohen. Control of the activity of highly purified cardiac actomyosin by Ca^{2+}, Na^{+}, and K^{+}. *Circulation Res.* 19: 1062–1070, 1966.
24. Lee, K. S., H. Ladinsky, and J. H. Stuckey. Decreased Ca^{2+} uptake by sarcoplasmic reticulum after coronary artery occlusion for 60 to 90 minutes. *Circulation Res.* 21: 439–444, 1967.
25. Mitchell, J. H., K. Wildenthal, and C. B. Mullins. Geometrical studies of the left ventricle utilizing biplane cinefluorography. *Federation Proc.* 28: 1334–1343, 1969.
26. Peterson, D. F., R. L. Kaspar, and V. S. Bishop. Reflex tachycardia due to temporary coronary occlusion in the conscious dog. *Circulation Res.* 32: 632–639, 1973.
27. Prinzmetal, M., L. L. Schwartz, E. Corday, R. Spritzler, H. C. Bergman, and H. E. Kruger. Studies on the coronary circulation. VI. Loss of myocardial contractility after coronary artery occlusion. *Ann. Internal Med.* 31: 429–449, 1949.
28. O'Rourke, R. A., B. Pegram, and V. S. Bishop. Variable effects of angiotensin infusion on left ventricular function. *Cardiovascular Res.* 6: 240–247, 1972.
29. Ruhmer, R. F., and D. K. Crystal. Changes in configuration of the ventricular chamber during the cardiac cycle. *Circulation.* 4: 211–218, 1951.
30. Stegall, H. F., M. B. Kardon, H. L. Stone, and V. S. Bishop. A portable, simple sonomicrometer. *J. Appl. Physiol.* 23: 289–293, 1967.
31. Stone, H. L., V. S. Bishop, and A. C. Guyton. Cardiac function after embolization of coronaries with microspheres. *Am. J. Physiol.* 204: 16–20, 1963.
32. Tatooles, C. J., and W. C. Randall. Local ventricular bulging after acute coronary occlusion. *Am. J. Physiol.* 201: 451–456, 1961.
33. Tennant, R., and C. J. Wiggers. The effect of coronary occlusion on myocardial contraction. *Am. J. Physiol.* 112: 351–361, 1935.
34. Tyberg, J. V., W. W. Parmley, and E. H. Sonnenblick. *In vitro* studies of myocardial asynchrony and regional hypoxia. *Circulation Res.* 25: 569–579, 1969.
35. Tyberg, J. V., L. A. Yeatman, W. W. Parmley, C. W. Urschel, and E. H. Sonnenblick. Effects of hypoxia on mechanics of cardiac contraction. *Am. J. Physiol.* 216: 1780–1738, 1970.

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Specificity of autonomic influences on cardiac responses during myocardial ischemia

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KASPAR, ROBERT L., GEORGE E. BARNES, D. FRED PETERSON AND VERNON S. BISHOP. *Specificity of autonomic influences on cardiac ... during myocardial ischemia.* J. Appl. Physiol. 38(3): 485–490. ... The possible role of the autonomic nervous system in the ... response to acute regional myocardial ischemia ... conscious dogs instrumented for measurement of ... pressure, internal diameter, and aortic flow. ... produced by occluding the left circumflex coronary ... tachycardia and reduced contractility. Changes ... control occlusions were compared with these during occlusions after beta-adrenergic blockade, parasympathetic blockade, and combined sympathetic and parasympathetic blockade. ... reduce the tachycardia and slightly reduced ... diameter changes in response to coronary occlusion. Results obtained in animals following surgical cardiac ... denervation indicated reduced tachycardia and no effects on ... contractile responses. The principal effect of parasympathetic blockade ... was an increase in end diastolic diameter during ... Right atrial pacing indicated this change was due to ... heart rates. Combined parasympathetic and sympathetic blockade did not alter inotropic responses to coronary occlusion. Results indicated that inotropic support due to changes in ... autonomic nerves is not increased during acute occlusion of the left circumflex coronary artery.

... reflexes, contractility; propranolol, atropine; ... rate, inotropic.

... SIGNIFICANCE of the autonomic nervous system ... regulating inotropic performance of the heart during myocardial ischemia in the conscious animal is unknown, although reflexes originating in or near the heart have been ... demonstrated in anesthetized animals. Studies have been ... to determine the involvement of peripheral ... vessels (10, 13), as well as changes in efferent neural activity in cardiac nerves (6, 7, 20) due to myocardial ischemia. Other studies have demonstrated changes in peripheral resistance (22), left ventricular dP/dt (1, 19), and heart rate (18) during electrical stimulation of afferent fibers in cardiac sympathetic nerves. Recent findings have implicated the autonomic nerves in regulation of heart rate during acute ischemia in conscious dogs (23).

In conscious dogs, acute myocardial ischemia results in an immediate diminution in left ventricular function (5). The response is characterized by progressive decreases in stroke volume, extent and rate of myocardial fiber shortening, and max dP/dt. Increases occur in the left ventricular end-diastolic pressure and heart rate. A new stable level of ventricular function, as assessed by the above variables, is reached in 30 s after the onset of the occlusion. The present study was designed to determine whether or not previously reported reflex changes in the autonomic neural activity to the heart during coronary occlusion in anesthetized animals (6, 7, 20) might be influencing the change in cardiac performance in conscious animals (5). One-minute occlusions of the left circumflex coronary artery were performed under control conditions, during parasympathetic blockade with atropine, beta-adrenergic blockade with propranolol, combined pharmacological blockade of both divisions of the autonomic nervous system, during atrial pacing, and after bilateral cardiac sympathectomy.

METHODS

Instrumentation

Sterile thoracotomies were performed under methoxyflurane anesthesia in 24 adult mongrel dogs (13–20 kg). In 15 animals, two sonomicrometer transducers were implanted across the greatest transverse diameter on the endocardial surface of the anterior and posterior left ventricular wall using techniques previously described (3, 4). Through a stab wound near the apex, a calibrated Konigsberg solid-state pressure transducer was implanted in the left ventricle. In 10 animals, an electromagnetic flow probe was placed around the ascending aorta. Polyvinyl catheters were placed in the left atrial appendage and into the carotid artery and jugular vein through a cervical incision. The left circumflex coronary artery was exposed and an occlusive device similar to that reported by Chimoskey et al. (9) was placed around it near its origin. Careful dissection minimized damage to the artery's nerve supply at the time of surgery. However, destruction of coronary nerves which may result in attenuation of coronary reflexes was possible. Intracircumflex coronary injections of Veratrum in animals which had not been instrumented and in animals instrumented with coronary flow probes and occluders demonstrated a depressor response in both groups. Thus, viable reflex pathways remain intact in spite of the risk of nerve damage during surgery. This was confirmed by direct electrical stimulation of the ansa subclavia in three animals immediately after instrumentation. To assure the effectiveness of our occluding device, in vivo transient occlusions were performed at the time of implantation and pressure required for occlusion noted. During postmortem

examination, the device was again checked. In animals containing coronary flowprobes, efficacy of the occlusive procedure was verified since it always eliminated blood flow in the artery within two to three seconds.

During the 2-wk recovery period, the health of the animals was monitored by hematocrit and body temperature examinations. All animals could exercise normally, and no electrocardiographic abnormalities were present.

Aortic flow was measured using a Zepeda SWFI electromagnetic flowmeter. The flow probes were calibrated in vitro before implantation and rechecked after the animals were sacrificed. In some cases, the in vivo calibration of the aortic flow probes was checked by using dye dilution techniques and, in all cases, the calibrations agreed within 5%. The signal in late diastole was assumed to represent zero aortic flow. Stroke volume was obtained by analog integration of the phasic aortic flow signal. Left ventricular transverse internal diameter was obtained using a sonomicrometer which measures the mean transit time for 5-mHz ultrasound between the two piezoelectric crystals at a sampling rate of 5,000 times/s. Since the velocity of sound in blood is known, transit time was convertible to distance. The sensitivity of the solid-state pressure transducer is stable in vitro and in vivo (16). Significant zero drift during implantation does occur from day to day; therefore, an independent zero reference was needed to set the pressure at the beginning of each experiment. Catheters inserted into the left ventricle under local anesthesia have, on occasion, been used for a reference and to confirm the in vivo calibrations. The catheter which measures *left ventricular* end-diastolic pressure was always within 1 mmHg of the mean left atrial pressure during control conditions (4, 16). We have routinely used the left atrial pressure during control conditions as a reference for the end diastolic pressure of the solid state pressure transducer. This method may affect the absolute levels of left ventricular pressure by as much as 1 mmHg but relative values during the experiment are correct (8).

Left atrial and arterial pressure were measured through Statham strain gauges (P23Db) zeroed to the midline of the sternum. Electrocardiograms were obtained from subcutaneous needle electrodes placed along the sternum. All signals were inscribed on type R Beckman oscillographic recorder. The first derivative of the diameter and pressure were recorded continuously at rest and during the occlusion of the circumflex coronary artery. Differentials were obtained using a SQ10A operational amplifier (Analogue Devices). The differentiator was calibrated using a triangular wave generator. The phase shift was 90° and the amplitude was linear between 0.5 and 100 Hz.

The Student *t*-test for paired observation was used for statistical analysis.

Experimental Interventions

Control. Resting measurements were obtained while the animal was lying quietly on its right side, unsedated and unrestrained. The left circumflex coronary artery was occluded for 1 min, then released. Following release of the occlusions, all parameters were allowed to return to the preocclusive level before additional occlusions were performed. This was usually accomplished within 90–120 s.

Autonomic blockade. Atropine (0.1 mg/kg) was used to produce parasympathetic blockade. Propranolol (0.5 to 1.0 mg/kg) was used to produce beta-adrenergic blockade. Effectiveness of the beta-blockade was assessed with a bolus of isoproterenol (4.5 μg) given prior to propranolol administration as well as several minutes afterward. If the response to isoproterenol was eliminated, the beta-adrenergic blockade was judged to be complete; if not, an additional 0.5 mg/kg of propranolol was administered. This always resulted in the elimination of the response to isoproterenol. Complete autonomic blockade was achieved by combining *atropine and propranolol in the doses described above*. One-minute occlusions of the left circumflex coronary artery were carried out on different days in order to examine specific effects of parasympathetic, beta-adrenergic, and combined blockade.

Cardiac sympathectomy. During the initial surgery, a loop of thread was placed around the left ansa subclavia of seven dogs. Sterile surgery was performed again 3 wk later in order to loop a second thread around the right ansa subclavia. Both threads were exteriorized at the back of the neck so that when pulled, the ansa subclavia would be cut, thereby eliminating the sympathetic innervation of the heart (21). The nerve section was always performed under general anesthesia. To eliminate the possibility of partial cardiac denervation resulting from the implantation of the aortic flow probe, all animals used in this portion of the study were not instrumented with flow probes on the ascending aorta.

Pacing. To determine the effect of heart rate alone, some animals underwent right atrial pacing via a bipolar pacing catheter passed intravenously via the jugular catheter into the right atrium. The pacing rate during occlusion was set to equal that achieved by the animal under parasympathetic blockade with atropine.

Critique of Method

Instrumentation. The frequency response of the sonomicrometer and left ventricular pressure transducer have been shown to be adequate (4). The transverse diameter as detected with the sonomicrometer has been shown to be a reliable index of changes in left ventricular volume under a variety of conditions which include alterations in preload, afterload, heart rate, and acute regional myocardial ischemia (2, 5). Asynchronous contractions during ischemia have been reported by numerous investigators (14, 15, 27, 28), suggesting that the sonomicrometer technique for relating diameter changes to volume changes might not always be reliable. However, as in previous studies, changes in transverse internal diameter were linearly related to volume ejected during control states and during the production of an ischemic region of the myocardium. Thus, even though asynchronous contractions may occur during ischemia, muscle shortening in the transverse plane must remain the major contributor to the volume ejected. Similar observations have been noted in clinical studies (11). Furthermore, the sonomicrometer transducers are not placed in cardiac muscle which is rendered ischemic during the occlusion. This has been confirmed by analysis of Silastic or latex injections into the circumflex coronary artery at autopsy as well as electrocardiographic changes

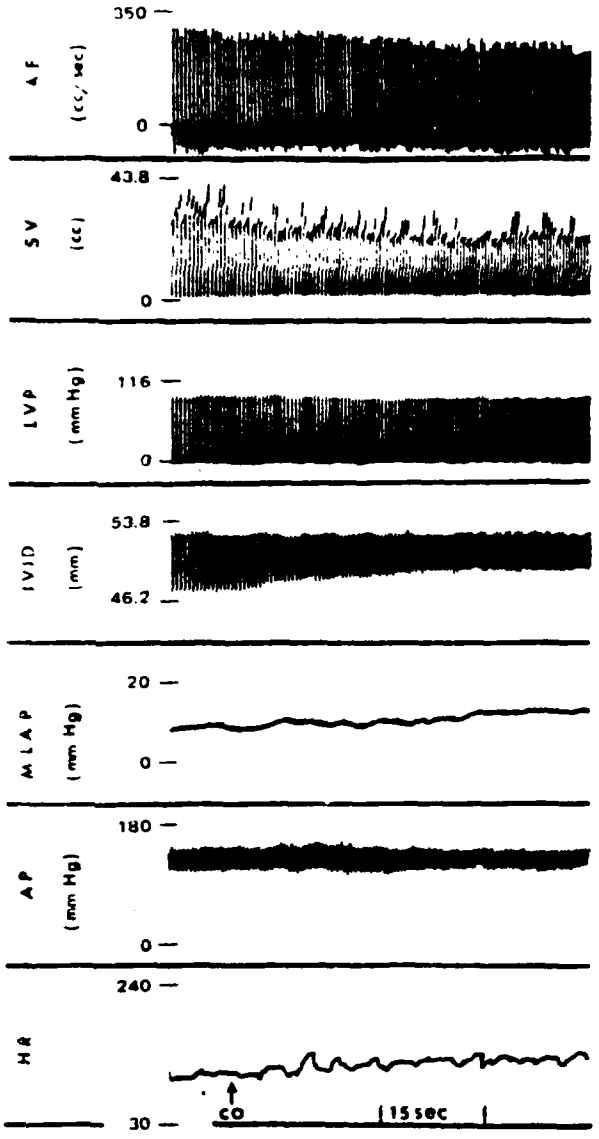

FIG. 1. Experimental records depicting the alterations in the measurement of left ventricular dynamics before and during coronary artery occlusion. Symbols are the same as in Table 1 except: MLAP, mean left atrial pressure; AF, pulsatile aortic flow; LVP, left ventricular pressure; CO, onset of coronary occlusion.

in acute dogs and histological and visual observations in animals with a permanently occluded circumflex coronary artery.

RESULTS

The changes in left ventricular function are illustrated by the changes in the various individual parameters in Fig. 1 and Table 1. During control occlusions, the most dramatic change was a rapid increase in the end-systolic diameter (ESD) (3.3 ± 0.3 mm) to a new constant level (Fig. 1). Since the end-diastolic diameter (EDD) was only slightly increased (0.8 ± 0.2 mm), the extent of shortening (EDD-ESD) was significantly reduced (−2.4 ± 0.4 mm). This reduction in extent of shortening parallels the decline in stroke volume (Fig. 1, Table 1). Left ventricular end-diastolic pressure and heart rate were increased respectively (6.5 ± 0.7 mmHg and 33 ± 4 beats/min) (Table 1). The maximum derivative of left ventricular pressure during the preejection phase (dP/dt) and the maximum derivative of diameter during ejection (dD/dt), which have been previously identified to be reliable indices of the inotropic state of the heart (2, 12) were significantly reduced (Table 1). The changes noted above agree with those previously reported during acute ischemia and indicate significant reductions in the extent and rate of myocardial fiber shortening (5, 11, 15).

When atropine was administered to conscious animals, the resultant vagal blockade caused significant increases in heart rate (116-174 beats/min). Stroke volume and EDD were reduced as a result of the tachycardia while ESD was not significantly changed (Table 1). This resulted

TABLE 1. *Drug effects and responses to coronary occlusion*

			Control	Atropine	Beta-Blockade
LVEDP, mmHg	R		3.8±0.5	2.2±0.5**	5.2±0.7*
	O (Δ)		6.5±0.7***	5.7±1.0	6.9±0.8
	N		24	14	24
HR, beats/min	R		116±3	174±5***	105±3*
	O (Δ)		33±4***	7±2†††	17±2†††
	N		24	14	24
AP, mmHg	R		97±3	111±3*	101±3
	O (Δ)		−10±2***	−13±2	−10±2
	N		17	12	17
LVID EDD, mm	R		29.6±1.3	−28.6±3.9**	30.8±1.4**
	O (Δ)		0.8±0.2***	2.0±0.4††	0.5±0.2††
	N		15	6	15
LVID ESD, mm	R		22.7±1.5	23.5±4.3	24.6±1.4***
	O (Δ)		3.3±0.4***	3.3±1.0	2.7±0.3†
	N		15	6	15
LVID ΔD, mm	R		6.9±0.5	5.2±0.6*	6.2±0.5*
	O (Δ)		−2.4±0.4***	−1.7±1.0	−2.1±0.3
	N		15	6	15
Max dD/dt, mm/s	R		58±4	57±10	49±4**
	O (Δ)		−12±3***	−21±5	−8±1
	N		15	6	15
Max dP/dt, mmHg/s	R		2741±153	2960±172	2237±215*
	O (Δ)		−476±63***	−403±88†	−322±108
	N		16	9	16
SV, ml/beat	R		24.6±2.2	18.1±1.7***	26.3±2.6
	O (Δ)		−8.2±0.7***	−4.6±0.9†††	−7.5±0.7
	N		10	6	10

Symbols: R, *resting value*; O (Δ), *maximum change during occlusion*; N, number of animals; LVEDP, left ventricular end diastolic pressure; HR, heart rate; AP, arterial pressure; LVID, left ventricular internal diameter; EDD, end-diastolic diameter; ESD, end-systolic diameter; ΔD, difference (EDD-ESD); max dD/dt, maximum derivative of diameter change during systole; max dP/dt, maximum derivative of pressure at the onset of systole; SV, stroke volume. Statistical comparisons with control resting values are indicated by *; comparisons with control changes during occlusion †. * or † $P < 0.05$. ** or †† $P < 0.01$. *** or ††† $P < 0.001$.

in a reduction in the extent of shortening, although dP/dt and dD/dt were relatively unaffected. These effects of heart rate during vagal blockade have been reported previously (3). Coronary occlusion during vagal blockade resulted in considerably greater increases in EDD (2.0 ± 0.4 mm) accompanied by significantly less fall in stroke volume (Table 1), while the heart rate response was substantially less (7 ± 2 beats/min) when compared with control responses. The responses of the other variables were similar to the control response although a small but significant inhibition in the fall in dP/dt during occlusion was observed (Table 1).

Since EDD was significantly increased due to coronary occlusion after atropine, the effects of atropine-induced tachycardia were examined. When the animals were free of drug interventions, heart rate was elevated by right atrial pacing to levels similar to those produced by atropine. Changes in EDD due to occlusion-induced ischemia during pacing were found to be the same as those after atropine administration (Fig. 2).

The left ventricular response to beta-adrenergic blockade is characterized by increases in filling pressure, EDD, and ESD. The increment in ESD may exceed the increment in EDD (3). In these cases, the extent of shortening and stroke volume are reduced. Arterial pressure is usually unaffected and heart rate may fall depending upon the initial rate. Small reductions occur in both phases of dP/dt and dD/dt.

After beta-adrenergic blockade, the left ventricular response to coronary artery occlusion was qualitatively similar to control. Responses included increases in EDD and ESD (0.5 ± 0.2 mm and 2.7 ± 0.3 mm, respectively), while the extent of shortening (EDD-ESD) was reduced (−2.1 ± 0.3 mm). Left ventricular end diastolic pressure was elevated 6.9 ± 0.8 mmHg and heart rate increased 17 ± 2 beats/min. The only significant alterations in the response to coronary ischemia during beta-adrenergic blockade were less dramatic changes in the EDD, ESD, and heart rate (Table 1). The effect on heart rate has been previously reported (23).

The reduction in the change in EDD and ESD seemed most likely related to the enlarged heart after beta-adrenergic blockade. This was investigated by studying seven animals which had undergone surgical cardiac sympathectomy after intact occlusion responses had been obtained. The only significant change in the resting values of the parameters

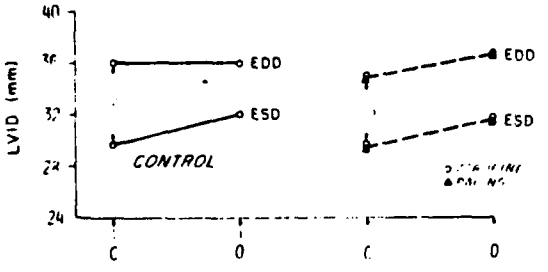

FIG. 2. Changes in end-diastolic and end-systolic diameter during coronary occlusion. Heart rate was elevated with atropine and by right atrial pacing. C, resting control value; O, maximum response to occlusion.

TABLE 2. *Responses to coronary occlusion before and after surgical sympathectomy*

		Control	Surgical Sympathectomy
LVEDP, mmHg	R	4.2±1.3	4.1±1.3**
	O (Δ)	6.1±1.4***	8.6±2.1
	N	7	7
HR, beats/min	R	98±6	87±7
	O (Δ)	26±11***	7±8†††
	N	7	7
AP, mmHg	R	97±4	98±4
	O (Δ)	−10±3***	−18±4†††
	N	7	7
LVID EDD, mm	R	33.1±3.6	32.5±3.7
	O (Δ)	0.8±0.2*	0.4±0.2
	N	4	4
LVID ESD, mm	R	27.0±3.6	26.2±3.9
	O (Δ)	2.5±0.5***	2.8±0.4
	N	4	4
SD LVID D, mm	R	6.1±2.1	6.3±3.6
	O (Δ)	−2.4±1.7***	−2.4±3.4
	N	4	4
Max dD/dt, mm/s	R	55±4.0	56±1.7
	O (Δ)	−10±1.9***	−10±2.2
	N	4	4
Max dP/dt, mmHg/s	R	8028±183	7934±161
	O (Δ)	−507±180***	−591±156
	N	7	7
SV, ml/beat	R	26.0±1.3	29.5±1.6
	O (Δ)	−7.9±0.8***	−8.2±1.1
	N	6	6

All symbols are the same as in Table 1.

measured after cardiac sympathectomy was an increase in left ventricular end diastolic pressure even though the absolute change was small. This was also observed after beta-blockade. As shown in Table 2, the inotropic responses to 1-min occlusion of the left circumflex coronary artery, after cardiac sympathectomy, were similar to those observed in intact trials. The notable exception was reduction in heart rate. As previously reported (23), surgical sympathectomy reduced tachycardia observed during coronary occlusion (Table 2). In addition, a slightly greater significant fall in arterial pressure was observed (Table 2). This was probably related to the dramatic reduction in tachycardia in the sympathectomized animals. Thus, the significant change in EDD and ESD during occlusion after propranolol was due to a secondary effect of the drug and not to a change in sympathetic neural influence (Table 1 and 2).

Since the basal metabolic state of the heart may be altered by propranolol or cardiac sympathectomy, the general inotropic responses to coronary occlusion were observed after combined parasympathetic and sympathetic blockade in four animals. Resting levels of dP/dt and dD/dt were lower than during control as observed after beta-blockade alone. Average changes were: dP/dt, 2,730–2,328 mmHg/s; dD/dt, 58–45 mm/s. Apparently, due to the elevated

heart rate (111 144 beats/min), resting EDD was smaller (28.2 25.6 mm) though ESD was not (20.3 20.3 mm), similar to that observed after atropine alone (Table 1). Blood pressure was elevated (98-110 mmHg). Since heart rate and blood pressure were substantially elevated by combined autonomic blockade, cardiac work presumably had been elevated above control levels as well. Coronary occlusion produced changes in dP/dt (-332 mmHg/min) and dD/dt (-13 mm/min) which were similar to those seen after propranolol alone (Table 1). As in the case of propranolol, the differences from control were not significant. Increases in EDD were greater than control ($+1.6$ mm) and ESD changes were similar to control ($+3.4$ mm). These responses are comparable to those observed after atropine alone.

DISCUSSION

The cardiovascular changes, which have previously been shown to occur during 1-min coronary occlusion of the left circumflex coronary artery in conscious dogs and which have been confirmed in this study, include immediate reductions in stroke volume, extent and rate of myocardial fiber shortening, and peak left ventricular pressure and its maximum derivative (5). Arterial pressure is slightly reduced and tachycardia develops in association with the elevation in mean left atrial pressure (5, 23). The tachycardia has been shown to be reflex in nature partially due to receptors in or near the heart with their efferent pathway carried primarily in the cardiac sympathetic fibers arising from the right stellate ganglion (23). Thus, changes that occur in response to acute myocardial ischemia initiate heart rate changes via the sympathetic nerves. However, due to the direct depressant effects of acute ischemia on the myocardium, previous studies have not attempted to investigate a possible supportive inotropic role for the autonomic nervous system which might be important in maintenance of cardiac function. Numerous investigations have described changes in efferent neural activity to the heart during coronary occlusion in the anesthetized cat (6, 7, 20) and small reflex inotropic changes have been observed during strong electrical stimulation of cardiac afferent nerves (1, 19). Those studies indicate the potential for reflexes arising from cardiopulmonary receptors; however, they do not indicate what physiological or pathological stimulus, if any, produces reflex inotropic physiological changes.

In the present study, the inotropic response to acute myocardial ischemia was essentially unaltered after beta-adrenergic receptor blockade or cardiac sympathectomy. The major difference observed was a change in the pre-occlusion resting values resulting directly from propranolol injection but which was not observed due to nerve section (Tables 1 and 2). Since the arterial pressure declines somewhat during ischemia, a reflex increase in the cardiac sympathetic activity would be expected (25). This has been shown to occur and contribute to tachycardia (23). If such an increase had affected the inotropic state of the heart, beta-adrenergic blockade would have been expected to alter the normal response to ischemia by extending the decrease in dP/dt, dD/dt, and the extent of shortening. This was not observed. Either propranolol or cardiac sympathectomy may depress the metabolic state of the heart. Yet, in these studies, it seems unlikely that the initial basal state of the heart was a factor in the inotropic changes observed during coronary occlusion. Administration of atropine plus beta-blockade elevated arterial pressure and heart rate above control but still did not alter the response to coronary occlusion observed after propranolol alone. Thus, the altered metabolic state of the heart did not appear to mask a sympathetic reflex neural influence which might support the inotropic state of the heart.

Malliani, Schwartz, and Zanchetti (20) have reported a cardiac reflex which is sometimes associated with inhibition of cardiac sympathetic efferent activity. Although not statistically significant, there was a definite attenuation in the average fall in dP/dt after beta-blockade in our experiments (Table 1) which was consistent in 13 of 16 dogs studied. This also appeared to be true in the four dogs treated with propranolol and atropine as well as for those animals treated with atropine alone. On the other hand, after cardiac sympathectomy, there was no change in the dP/dt response observed. Thus, the attenuation in the fall in dP/dt appears to be associated with drug effects and is not related to changes in sympathetic efferent activity.

The major difference observed between control occlusion and occlusion after parasympathetic blockade was that during occlusion with parasympathetic blockade, there was a much larger change in the EDD. Such a large change in EDD after atropine, pacing or beta-blockade plus atropine may seem puzzling but was apparently related to the higher heart rates. A probable explanation is related to the resting length-tension relationship of the myocardium. Sarnoff and Mitchell (26) have shown that, at shorter muscle lengths, the muscle has a low resting tension and a given amount of pressure will stretch it considerably. At longer muscle lengths, the muscle has a higher resting tension and the same amount of pressure stretches it a smaller amount. The increased heart rate following parasympathetic blockade reduced the EDD and the resting tension of the myocardium. During occlusion, the increase in filling pressure led to a greater increase in EDD and slightly less reduction in dP/dt. Identical responses to ischemia were noted when heart rate was initially elevated by right atrial pacing. Thus, the increment in EDD during acute myocardial ischemia depends upon the resting tension which in turn will be modified by changes in the resting heart rate.

This study confirms the finding that reflex tachycardia during acute ischemia is much reduced by cardiac sympathectomy or beta-adrenergic blockade (23). However, the contractile changes as measured by dP/dt and dD/dt were not affected by these neural interventions. This suggests one of two possible conclusions. First, the heart may be so depressed, mechanically, by the ischemia that it is unable to respond to the sympathetic inotropic input with an increase in our measured parameters. This explanation does not appear likely since direct electrical stimulation of the ansa subclavia or isoproterenol infusion during coronary occlusion in anesthetized dogs causes rapid, dramatic increases in left ventricular dP/dt (17; personal observation). Direct electrical stimulation of the ansa subclavia

after propranolol (1 mg/kg) abolished the previously described response (personal observation). In fact, if the heart had been mechanically depressed and unresponsive to sympathetic input, one would expect that the fall in dP/dt would be greater during occlusions after propranolol had been given. This was not the case. The second possibility is that the heart is able to respond but no increase in sympathetic inotropic support occurs. Such a possibility could indicate that under our experimental conditions, either the threshold for inotropic change is higher than that for chronotropic change or there is separation of functional sympathetic neural inputs to the heart. The latter has been demonstrated (12, 23, 24).

One is left with the conclusion that, in this study, there was no measurable increase in cardiac sympathetic inotropic activity. This conclusion is teleologically appealing since it would seem to indicate that the system is acting to prevent increased work and, thus, avoid increased O_2 demands by the heart.

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REFERENCES

1. ARMOUR, J. A., AND W. C. RANDALL. A cardio-cardiac reflex in the dog (Abstract). *Federation Proc.* 32: 331, 1973.
2. BARNES, G. E., V. S. BISHOP, L. D. HORWITZ, AND R. L. KASPAR. The maximum derivatives of left ventricular pressure and transverse internal diameter as indices of the inotropic state of the left ventricle in conscious dogs. *J. Physiol., London* 235: 571–590, 1973.
3. BISHOP, V. S., AND L. D. HORWITZ. Effects of altered autonomic control on left ventricular function in conscious dogs. *Am. J. Physiol.* 221: 1278–1282, 1971.
4. BISHOP, V. S., L. D. HORWITZ, H. L. STONE, H. F. STEGALL, AND E. J. ENGELKEN. Left ventricular internal diameter and cardiac function in conscious dogs. *J. Appl. Physiol.* 27: 619–623, 1969.
5. BISHOP, V. S., R. L. KASPAR, G. E. BARNES, AND M. B. KARDON. Left ventricular function during acute regional myocardial ischemia in the conscious dog. *J. Appl. Physiol.* 37: 785–792, 1974.
6. BROWN, A. M. The depressor reflex arising from the left coronary artery of the cat. *J. Physiol., London* 184: 825–830, 1966.
7. BROWN, A. M., AND A. MALLIANI. Spinal sympathetic reflexes initiated by coronary receptors. *J. Physiol., London* 212: 685–705, 1971.
8. BRAUNWALD, E., AND C. J. FRAM. Studies on Starling's law of the heart. IV. Observations on the hemodynamic function of the left atrium. *Circulation* 24: 633–642, 1961.
9. CHIMOSKEY, J. E., M. SZENTIVANYI, R. ZAKHEIM, AND A. C. BARGER. Temporary coronary occlusion in conscious dogs: collateral flow and electrocardiogram. *Am. J. Physiol.* 212: 1025–1032, 1967.
10. COSLANTIN, L. Extracardiac factors contributing to hypotension during coronary occlusion. *Am. J. Cardiol.* 11: 205–217, 1963.
11. DWYER, E. M., JR. Left ventricular pressure-volume alterations and regional disorders of contraction during myocardial ischemia induced by atrial pacing. *Circulation* 42: 1111–1122, 1970.
12. FURNIVAL, C. M., R. J. LINDEN, AND H. M. SNOW. Reflex effects on the heart of stimulating left atrial receptors. *J. Physiol., London* 218: 447–463, 1971.
13. HANLEY, H. G., J. C. COSTIN, AND N. S. SKINNER, JR. Differential reflex adjustments in cutaneous and muscle vascular beds during experimental coronary artery occlusion. *Am. J. Cardiol.* 27: 513–521, 1971.
14. HERMAN, M. V., AND R. GORLIN. Implications of left ventricular asynergy. *Am. J. Cardiol.* 18: 556–573, 1966.
15. HOOD, W. B., JR., V. H. COVELLI, W. H. ABELMANN, AND J. C. NORMAN. Persistance of contractile behavior in acutely ischemic myocardium. *Cardiovascular Res.* 3: 249–260, 1969.
16. HORWITZ, L. D., AND V. S. BISHOP. Left ventricular pressure-dimension relationships in the conscious dog. *Cardiovascular Res.* 6: 163–171, 1972.
17. KERBER, R. E., F. M. ABBOUD, M. L. MARCUS, AND D. L. EICHBERG. Effect of inotropic agents on the localized dyskinesis of acutely ischemic myocardium. *Circulation* 49: 1038–1046, 1974.
18. MALLIANI, A., M. PARKS, R. P. TUCKFIELD, AND A. M. BROWN. Reflex increases in heart rate elicited by stimulation of afferent cardiac sympathetic nerve fibers in the cat. *Circulation Res.* 32: 9–14, 1973.
19. MALLIANI, A., D. E. PETERSON, V. S. BISHOP, AND A. M. BROWN. Spinal sympathetic cardio-cardiac reflexes. *Circulation Res.* 30: 458–466, 1972.
20. MALLIANI, A., P. J. SCHWARTZ, AND A. ZANCHETTI. A sympathetic reflex elicited by experimental coronary occlusion. *Am. J. Physiol.* 217: 703–709, 1969.
21. MIZERES, N. J. The origin and course of the cardioaccelerator fibers in the dog. *Anat. Record* 132: 261–279, 1959.
22. PETERSON, D. F., AND A. M. BROWN. Pressor reflexes produced by stimulation of afferent cardiac nerve fibers in the cardiac sympathetic nerves of the cat. *Circulation Res.* 28: 605–610, 1971.
23. PETERSON, D. F., R. L. KASPAR, AND V. S. BISHOP. Mechanism of reflex tachycardia due to coronary occlusion in the conscious dog. *Circulation Res.* 32: 652–659, 1973.
24. RANDALL, W. C., J. A. ARMOUR, W. P. GEIS, AND D. B. LIPSCOMB. Regional cardiac distribution of the sympathetic nerves. *Federation Proc.* 31: 1199–1208, 1972.
25. SARNOFF, S. J., J. P. GILMORE, S. K. BROCKMAN, J. H. MITCHELL, AND R. J. LINDEN. Regulation of ventricular contraction by the carotid sinus. Its effect on atrial and ventricular dynamics. *Circulation Res.* 8: 1123–1136, 1960.
26. SARNOFF, S. J., AND J. H. MITCHELL. The control of the function of the heart. In: *Handbook of Physiology. Circulation.* Washington, D.C.: Am. Physiol. Soc., 1962, sect. 2, vol. 1, chapt. 15, p. 489–532.
27. TATOOLES, C. J., AND W. C. RANDALL. Local ventricular fatigue after acute coronary occlusion. *Am. J. Physiol.* 201: 451–456, 1961.
28. TENNANT, R., AND C. J. WIGGERS. The effect of coronary occlusion on myocardial contraction. *Am. J. Physiol.* 112: 351–361, 1935.

Reflex Heart Rate Control Via Specific Aortic Nerve Afferents in the Rabbit

By **Merrill B. Kardon, D. Fred Peterson, and Vernon S. Bishop**

ABSTRACT

Reflex bradycardia was elicited in rabbits via repetitive electrical stimulation of the central end of the sectioned left aortic nerve. Supramaximal stimulation produced a 16.9 ± 1.3% (SE) increase in the R-R interval when vagal and sympathetic efferent pathways were intact. Reducing the stimulation voltage allowed selective stimulation of the myelinated (A) fibers, and polarizing electrodes placed central to the stimulus site permitted A fiber blockade and selective stimulation of the unmyelinated (C) fibers. When afferent A fibers were selectively stimulated, 64% of the maximum response was obtained; selective C fiber activation elicited 63% of the maximum observed response. Selective stimulation of A or C fibers after either vagotomy or stellectomy indicated that A fiber afferents elicit heart rate responses via both vagal and sympathetic efferents, whereas C fiber afferent information is mediated predominantly via vagal efferents. This afferent-efferent specificity of the aortic baroreceptor pathways suggests baroreceptor mechanisms normally used to modulate heart rate. Small increments in blood pressure would activate low-threshold A fibers and result in reciprocal changes in vagal and sympathetic efferent activity. More substantial increases in blood pressure would activate afferent C fibers and produce additional heart rate effects via vagal efferents.

■ The influence of baroreceptors on heart rate has been shown to involve afferent myelinated (1) and unmyelinated (2) fibers. Central stimulation of the rabbit aortic nerve causes a reduction in both heart rate and arterial blood pressure (2–4). The mechanisms whereby each of the aortic nerve afferent fiber groups cause reflex changes in heart rate have not been well defined. Recent data indicate that unmyelinated afferent (C) fibers, which have been shown to arise in the wall of the aortic arch (5), may carry a significant portion of the overall baroreceptor information in the rabbit (2) as well as in the dog and the cat (6).

Previous investigators have shown that as arterial blood pressure is elevated reflex peripheral vascular resistance changes occur at a lower pressure than do heart rate changes (7–9). This finding indicates the possibility that a functional separation of baroreceptor-induced vagal and sympathetic cardiovascular influences may be attributable to the differences in their respective afferent mechanisms. Although reflex heart rate effects have been shown to involve reciprocal changes in cardiac vagal and sympathetic efferent activity (10), the specific afferent fiber groups responsible for these reciprocal changes have not been identified. The present study was designed to evaluate the ability of the rabbit's myelinated (A) and unmyelinated (C) aortic nerve groups to vary heart rate by way of either vagal or sympathetic efferent pathways.

Methods

Twenty-three albino rabbits, 1.5–2.5 kg, were anesthetized with sodium pentobarbital (30 mg/kg, iv). Catheters were placed in the femoral vein so that anesthetic could be administered and the thoracic aorta arterial blood pressure could be monitored with a Statham P23db transducer. A midventral cervical incision was made from the point of the sternum to the angle of the jaw, and artificial respiration was begun using a Harvard Apparatus ventilator (model 665) at a volume of 30 ml and a rate of 100 /min. The low-volume, high-frequency air flow produced synchronization (with the respirator) of the rabbit's spontaneous respiratory movements; minimum reflex changes in arterial blood pressure were observed. This technique has been shown to cause little, if any, qualitative change in baroreceptor-mediated responses (11) or in blood gas levels (2, 12). A pouch was formed by suspending the free ends of the incised skin from a horizontal ring in place above the incision. Using a binocular dissecting microscope (Olympus model SZ), the left aortic nerve was dissected free in the neck and cut as close as possible to the sternum. Insofar as A fibers respond at lower electrical thresholds than do C fibers, it was possible to stimulate A fibers selectively by reducing the stimulus voltage from supramaximal levels to sub-C fiber threshold levels. The reverse, however, was not

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possible. To elicit the preferential activation of C fibers, the conduction of A fibers was first blocked using a pair of polarizing electrodes (13). Three sites were isolated along the length of the left aortic nerve. The free central end of the cut nerve was placed on a pair of platinum-irridium stimulating electrodes connected by way of a Grass SIU5 stimulus isolation unit to one channel of a Grass model S-88 stimulator. At the second more rostral site midway along the length of the left aortic nerve in the neck, a second pair of electrodes was positioned. These electrodes were covered with saline-soaked cotton wicks and connected via a second stimulus isolation unit to the 100,000-ohm d-c output of the second channel of the S-88 stimulator. As previously described, they served as A fiber blocking electrodes (13). Bipolar recording electrodes were placed at the third most rostral site. These electrodes were connected to a pair of cascaded Grass model P15 preamplifiers, and they served to monitor the evoked potential. The evoked potential was recorded on Polaroid film from the face of a Tektronix D12 storage oscilloscope. Bilateral vagotomies were performed in the cervical region. Stellectomies were performed by removing a section of the sympathetic chain between the first and the third thoracic interspace. Heart interval was monitored by way of needle electrodes placed along the sternum. These electrodes were connected to a Beckman type 9857B cardiotachometer coupler. Both heart rate and arterial blood pressure were recorded continuously using a Beckman type R-M (411) oscillograph.

On-line monitoring of the R-R interval and control of the nerve stimulus parameters were accomplished using a DEC PDP 8/E digital computer. Bursts of stimulus impulses were applied to the left aortic nerve during each R-R interval in fixed synchrony with each R wave. The duration of each impulse was 0.3 msec as established by the stimulator. The run duration and the stimulation sequence, which consisted of impulse frequency, impulse number, burst duration, and timing of the stimulus burst within each R-R interval, were established by the computer in accordance with the experimental protocol. The computer stored each R-R interval in a given run and averaged it with the equivalent interval in previous runs made under identical stimulus conditions. The sequence of average R-R interval values for each group of stimulus-response runs was then printed out on command. Three key parameters, latency to onset, latency to peak, and the value of the peak response were printed out along with their respective standard deviations. The latency to onset represents the number of heart intervals from the beginning of the stimulus to the end of the interval which first exceeded the control. The latency to peak represents the number of heart intervals from the beginning of the stimulus to the end of the longest interval during the response. Peak response represents the maximum percent change from the control R-R interval.

Comparisons were made between the magnitude of the reflex bradycardia elicited by either A or C fiber stimulation before and after selective deefferentation (vagotomy or stellectomy). To activate aortic afferent A fibers alone, the stimulus intensity was adjusted while the evoked potential was monitored. When the stimulus voltage was reduced enough to eliminate activation of C fiber afferents, while causing little or no change in A fiber activation (Fig. 1A), a stimulus-response run was begun. In a separate group of rabbits, the C fiber afferents were activated to the exclusion of the A fibers by using the technique of Manfredi (13). While the left aortic nerve was stimulated at supramaximal intensity (sufficient to activate all fibers), a small blocking current (5–15 μa) was applied to the nerve via the interposed blocking electrodes. By adjusting the blocking current while the evoked potential was monitored, it was possible to eliminate A fiber conduction and leave C fiber conduction little affected (Fig. 1B). A stimulus-response run could then be made while only C fibers were being

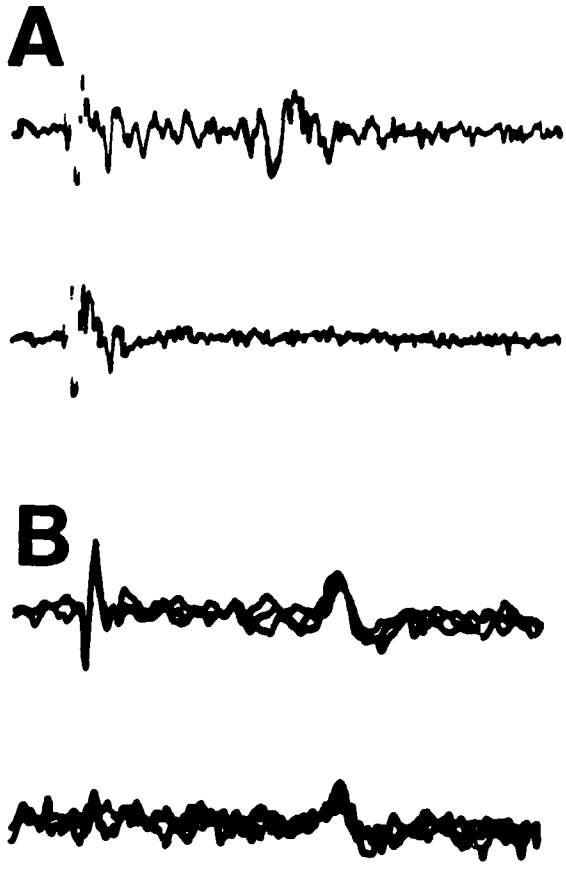

FIGURE 1

A: *Single stimulus compound-evoked potential recorded from the left aortic nerve.* Top: *At a supramaximal stimulus intensity, A fibers (fast component at left) and C fibers (slow wave at center) are activated.* Bottom: *At a reduced stimulus voltage, C fibers are no longer activated, but the A wave is unchanged. Interelectrode distance = 30 mm, sweep speed = 5 msec/cm.* **B:** *Overlay of three single stimulus compound-evoked potentials which were recorded in rapid succession illustrating blockage of A fiber conduction.* Top: *At a supramaximal stimulus intensity all fibers are activated.* Bottom: *When the blocking current (5–15 μa), is on, the A wave is blocked; the C wave is reduced slightly. Interelectrode distance = 35 mm, sweep speed = 5 msec/cm. The horizontal bar represents 1 cm of the oscilloscope sweep or 5 msec in time.*

stimulated. Without left aortic nerve stimulation, the blocking current never elicited a persistent change in either arterial blood pressure or heart rate. Subsequent to the control runs, selective deefferentations were performed (bilateral vagotomy or bilateral stellectomy). Repeat runs were carried out to indicate the relative importance of vagal and sympathetic pathways in the control of heart rate via either myelinated or unmyelinated aortic afferents.

Standard errors were calculated for the onset and peak latencies as well as for the peak responses. The significance of the difference was determined by the *t*-test. P values < 0.05 were considered significant.

CRITIQUE OF METHODS

The polarizing blocking technique used in this series of experiments has been shown to cause selective blockade of myelinated fiber conduction by producing a failure of conduction between the blocking anode and cathode (13). The A beta group is most sensitive to the d-c blockade. However, at current levels sufficient to block some of the beta group, asynchronous firing of some less sensitive nerve fibers occurs. This phenomenon has been shown to result from cathodal excitation. With complete blockade of the beta fibers (and subsequent blockade of the delta group as well), the asynchronous firing no longer is seen at the recording site due to conduction failure at the anode. The conduction of unmyelinated C fibers is only slightly affected by this level of current.

In the present experiments, the direct current was adjusted to block myelinated fiber conduction while causing as little asynchronous firing as possible. However, insofar as d-c levels that completely suppressed synchronous firing also caused significant C wave depression, the level was adjusted to yield A wave blockade with as little C wave depression as possible. The amount of asynchronous firing passing the blocking anode was judged to have little qualitative effect on the observed response. During the course of the electrical nerve block, the blocking current often fell somewhat during the first 15–30 seconds, no doubt as a result of electrode polarization during the blocking phase. This fall sometimes led to a partial loss of block selectivity. However, a run was not made until the block was reestablished (by raising the blocking current slightly) and its stability verified for at least 30 seconds. Subsequently, during the blocking period, no detectable blocking current creep was seen nor was there a significant tendency for the block to degrade. Selective nerve block was not normally maintained for periods longer than 2 minutes. The current level necessary to effect a selective aortic nerve block was essentially unchanged for all trials in each rabbit. Barbiturates are well known for their ability to depress the vagal centers. However, in our experiments, the rabbits were maintained at a light surgical anesthetic level with little apparent depression of the vagal component as indicated by the slope of the heart rate response.

Results

In 23 rabbits, repetitive synchronous stimulus bursts were applied to the left aortic nerve at frequencies of 50 and 100 Hz. In 13 rabbits, a total of 48 trial runs was made while stimulating at supramaximal intensity (all aortic nerve fibers activated) (Fig. 2). The average R-R interval increased 16.9% from the control level of 209 ± 3 msec. The latency to onset and that to peak bradycardia were 4.4 intervals and 21.0 intervals, respectively. Bilateral vagotomy had no significant effect on resting heart rate, as previously reported (2). It did result in a reduction of the peak response to 11.0%. The latency to onset and the latency to peak were increased to 8.4 and 32.6 intervals,

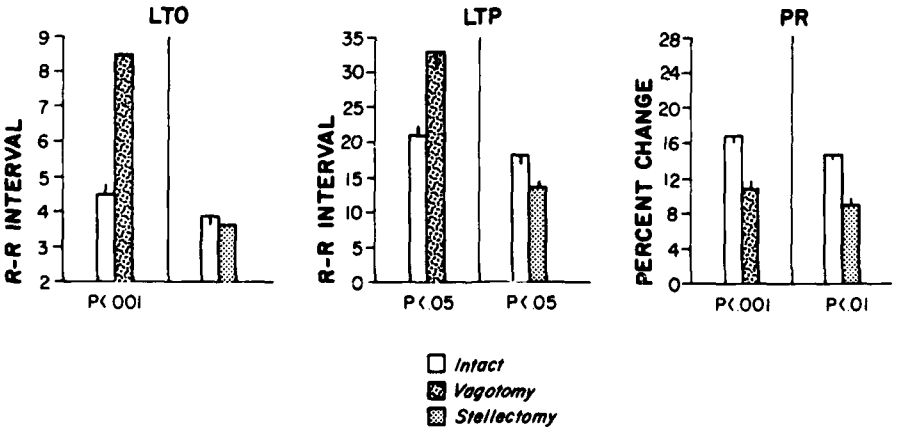

FIGURE 2

Influence of efferent neural pathways on the heart rate response during supramaximal aortic nerve stimulation. Each pair of bars compares responses after either vagotomy or cardiac sympathectomy to responses obtained immediately prior to nerve section. Latency to onset (LTO) *and latency to peak* (LT^P) *are both measured in R-R intervals after initiation of electrical stimulation. Peak response* (PR) *is measured by the percent change in the R-R interval when the heart has slowed maximally.*

respectively. Thus, vagotomy reduced the peak response by 35% ($P < 0.001$), while the latencies to onset and to peak were significantly delayed in time. In the remaining 10 rabbits, 33 trial runs produced an average bradycardia of 14.9%. The onset and the peak bradycardia occurred at 3.8 and 17.9 intervals, respectively, from the stimulus onset. Stellectomy slightly decreased the resting heart rate and reduced the reflex bradycardia by 41% ($P < 0.01$). The latency to onset was not significantly affected, and the peak bradycardia occurred 4.5 intervals sooner ($P < 0.05$). Thus, when the entire aortic nerve was activated, loss of the sympathetic efferent pathways reduced the magnitude of the reflex bradycardia, and the latency to peak response but had no effect on the latency to onset. In contrast, loss of the parasympathetic pathways extended both the onset and the peak latency while it, too, reduced the magnitude of bradycardia.

The relative influence of afferent A and C fiber activation on heart rate response, when all efferent pathways are intact, was studied in 11 rabbits (Fig. 3). Activation of A fibers alone caused no significant change in the latency to onset. When C fibers were selectively activated, the latency to onset was increased from a control value of 4.6 intervals to 6.0 intervals, which was a significant 31% increase ($P < 0.01$). The latency to peak was greatly reduced when C fibers were not activated. The reduction observed was 19.7 intervals to 11.4 intervals ($P < 0.01$). When C fibers alone were activated, there was a reduction from control, 19.6 intervals to 15.3 intervals, which was not significant. The peak response was equally dependent on activation of both A and C fiber afferents. The reduction in peak response was from 19.0% to 12.2% ($P < 0.02$) when afferent A fibers were stimulated alone, whereas stimulation of C fibers alone reduced the response from 13.4% to 8.0% ($P < 0.01$).

In 11 rabbits, a comparison was made between the reflex heart rate response to A fiber activation before and after either vagotomy or stellectomy (Fig. 4). Selective A fiber stimulation produced an average fall (peak response) in heart rate of 10.5%. The latencies to onset and peak were 4.1 and 11.0 intervals, respectively. Vagotomy in 6 of these rabbits reduced the peak response by 63%, and the onset of the response was delayed by 3.0 intervals, ($P < 0.05$). In the remaining 5 rabbits, stellectomy reduced the peak response to 5.4% ($P < 0.01$) (a 61% reduction). The latencies to onset and peak were 4.8 and 10.2 intervals, respectively, but neither of these changes was statistically significant.

In ten rabbits, A fiber conduction was blocked, and a total of 30 trial runs was performed (Fig. 5). In trials performed in six of these rabbits, C fiber stimulation reduced heart rate by 8.5%. The latencies to onset and peak were 5.8 and 16.5 intervals, respectively. Vagotomy in this group reduced the peak response to 4.9% and increased the latencies to onset and peak to 8.8 and 22.8 intervals, respectively. Thus, vagotomy reduced the peak response by 42% ($P < 0.01$). The onset of the response was delayed by 3.0 intervals ($P < 0.05$). In a total of 11 trial runs performed in the remain-

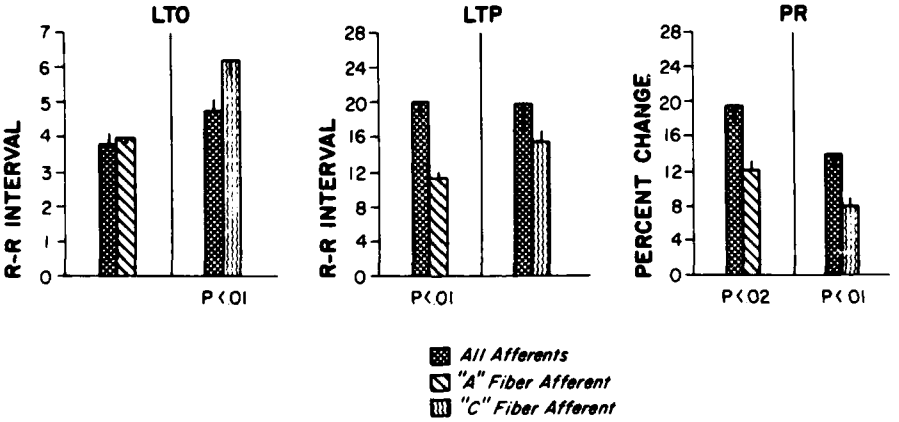

FIGURE 3

Afferent aortic nerve fiber influences on reflex bradycardia. Each pair of bars compares either A fiber or C fiber stimulation with supramaximal stimulation carried out immediately prior to selective stimulation. Comparison of A fiber stimulation with control represents a mean of 31 averaged trials in 11 rabbits. C fiber comparisons with control are for 30 trials in 10 rabbits. Abbreviations are the same as they are in Figure 2.

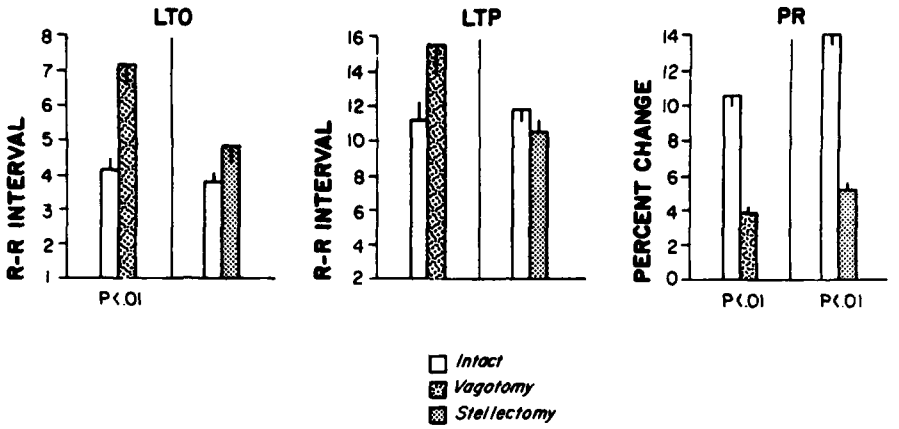

FIGURE 4

Influence of efferent neural pathways on the heart rate response during selective aortic A fiber stimulation. Each pair of bars compares responses after either vagotomy or cardiac sympathectomy with responses prior to nerve section. Definitions are the same as they are in Figure 2.

ing four rabbits, stellectomy did not cause a significant change in peak response. No significant change was observed in either latency to onset or the latency to peak. When aortic unmyelinated fibers were activated, loss of parasympathetic efferent pathways caused a reduction in peak effect and a delay in both onset and peak bradycardia. Loss of sympathetic efferents, however, had no significant effect on the peak response, indicating that aortic nerve unmyelinated fibers mediate heart rate predominantly via vagal efferents.

Discussion

This study provides the first direct evidence that aortic nerve-mediated reflex bradycardia occurs in response to activation of either afferent A or C fibers and that its magnitude depends on the number and the type of fibers being activated.

The effect of A and C fiber afferents as well as that of vagal and sympathetic efferents on the peak response indicates that A and C fiber afferents are approximately equipotent so long as both efferent pathways are intact. Vagal and sympathetic efferent pathways are equally important in eliciting a reflex bradycardia when all fibers or when only the A fibers are activated. However, removal of the sympathetic efferents has no effect on the heart rate response when only C fibers are activated. Since the bradycardia to selective C fiber stimulation is not abolished after vagal section, we cannot unequivocally disregard a sympathetic efferent involvement in the reflex response. Possibly, in the

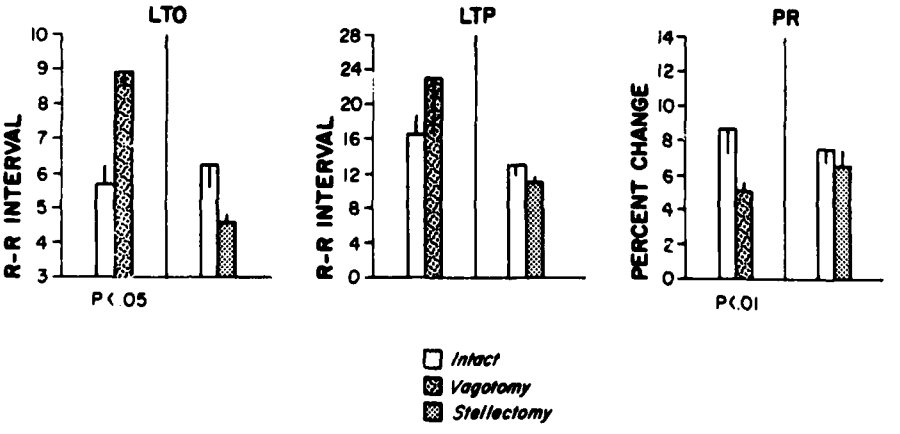

FIGURE 5

Influence of efferent neural pathways on the heart rate response during selective aortic C fiber stimulation. Each pair of bars compares responses after either vagotomy or stellectomy with responses prior to nerve section. Definitions are the same as they are in Figure 2.

presence of intact vagal influences, sympathetic influence is minimal, whereas in the absence of vagal activity sympathetic influences are substantial during selective aortic C fiber stimulation. Similar responsiveness has been demonstrated in isolated atria exposed to acetylcholine and norepinephrine (14). In any case, insofar as baroreceptor effects on heart rate are concerned, the cardiac sympathetic influence appears to be primarily dependent on changes in afferent A fiber activity. In contrast, vagal influences are modulated by both afferent A and C fibers in the aortic nerve. These results are supported by similar findings during carotid sinus stimulation and neural physiological studies of the vasomotor areas of the brainstem (15, 16). In the brainstem of the cat and the rabbit, closely situated areas have been identified which receive information from either A or C fibers (15, 16). Furthermore, Kumada and Nakajima (15) have demonstrated that inputs from myelinated aortic nerve afferents innervate both the vagal and the sympathetic sensory areas of the rabbit brainstem and that unmyelinated afferents are absent from the sympathetic sensory areas.

Selective activation of either A or C fiber afferents demonstrates that the myelinated group is responsible for the earliest onset of bradycardia. This finding, of course, is predictable, since myelinated fibers can have many times the conduction velocity of unmyelinated fibers. Likewise, selective cardiac efferent nerve section demonstrates that intact vagi are essential to elicit the earliest onset of reflex bradycardia. Again, this finding is predictable, since the vagus contains many myelinated efferent fibers (17, 18) and the cardiac sympathetic nerves contain few myelinated fibers of any type (19, 20).

Our results illustrate the long time constant nature of the sympathetic influence on heart rate. The elimination of efferent cardiac sympathetic nerves causes a reduction in the number of intervals to peak effect with no significant effect on the latency to onset. This finding infers that rapidly conducting A fiber afferents which modify sympathetic efferent activity serve a function which is not dependent on their high conduction velocity. However, rapid modification of heart rate is vagally mediated (10). This study shows that myelinated aortic nerve fibers influence the vagal control of heart rate. Consequently, the fastest potential aortic baroreceptor influence on heart rate must occur by way of these afferent A fibers modulating efferent vagal activity. The efferent vagal fiber types which are modulated by these aortic afferents are not known at this time. However, working in the cat, Kunze (17) has shown that activation of the arterial baroreceptors causes demonstrable changes in the activity of vagal efferents which innervate the sinoatrial node. These vagal efferents are small-diameter myelinated fibers.

An indication of the order of activation of the A and C fiber aortic baroreceptor pool may be inferred from the known progression of peripheral vascular resistance and heart rate effects seen with increasing arterial blood pressure. Glick and Covell (8) and Allison et al. (7) have shown that with increasing arterial blood pressure in the dog, reflex reduction in peripheral vascular resistance (which is assumed to be largely under the influence of the sympathetic nervous system) occurs at a lower blood pressure threshold than does bradycardia. This finding indicates that during the course of an increase in arterial blood pressure the afferents which are activated at lower pressures are those which tend to suppress sympathetic efferent activity to the vasculature. Angell-James (21) has shown that, when they are activated physiologically, aortic nerve fibers fire at a frequency which is largely independent of the rate of change of pressure in the aortic arch so long as the pressure is above threshold for a given receptor. This finding suggests that the relative importance of recruitment of additional baroreceptor afferents as pressure increases is greater than that of increasing firing rates of individual fibers during the systolic phase. The results of Kardon et al. (3) demonstrate the importance of impulse number to aortic nerve reflex heart rate effects. These two studies, in combination with the present work, indicate that the important features of rabbit baroreceptor control of heart rate are the type and the number of fibers being activated over a given time period as well as their individual thresholds. If aortic C fibers have different pressure thresholds than do aortic A fibers, the reflex effect of C fiber recruitment on heart rate should differ from the reflex effect of recruitment of additional A fibers, as shown in this study. Therefore, increases in the frequency of whole aortic nerve activity in response to augmentation of arterial blood pressure must occur largely as a result of successive recruitment of nerve fibers having higher pressure thresholds rather than by increases in the firing rates of the individual nerve fibers involved. Finally, a previous study (2) has shown a progressive increase in reflex effects with increased recruitment of aortic nerve afferents: blood pressure effects are seen at the lowest stimulus intensities (rapidly conducting A fiber activa-

tion), but heart rate effects require recruitment of more slowly conducting myelinated fibers. Thus, it is inferred that the magnitude of the heart rate response can be augmented until all afferent C fibers are activated as well.

In combination with what has been shown in the present study to be the afferent-efferent specificity of the aortic baroreceptor pathways, the order of electrical activation of the afferent fibers from myelinated to unmyelinated fibers may illustrate the baroreceptor mechanisms normally used to modulate heart rate. If the A fiber pool with its combined vagal-stimulatory and sympathoinhibitory effects is activated at lower blood pressure thresholds, the normal baroreceptor control of heart rate would occur via their reciprocal action on vagal and sympathetic efferents at small increments in arterial blood pressure. More sustained increases in blood pressure would activate vagal efferents in larger proportion via C fiber afferents and would produce more profound heart rate effects.

Sympathetic regulation of heart rate in a predominantly high-frequency, low-threshold baroreceptor system suggests th these sympathetic efferents are modulated by subtle changes in arterial blood pressure. The known frequency-response characteristics of the sympathetic neuroeffector mechanism no doubt cause this subtle reflex bradycardia to occur over a longer time course than does that initiated by changes in vagal activity. Fluctuations in blood pressure which alter sympathetic influences via afferent A fibers would certainly cause rapid beat-to-beat changes in heart rate via vagal efferents. When blood pressure exceeds this range, the increased recruitment of C fiber afferents would cause the potential for beat-to-beat heart rate control to be progressively masked. Under these circumstances, heart rate would no doubt fall farther in response to continuous activation of both A and C fiber afferents and vary less in response to beat-to-beat blood pressure changes.

References

1. PELLETIER CL, CLEMENT DL, SHEPHERD JT: Comparison of afferent activity of canine aortic and sinus nerves. Circ Res **31**:557-568, 1972
2. KARDON M, PETERSON DF, BISHOP VS: Reflex bradycardia due to aortic nerve stimulation in the rabbit. Am J Physiol **225**:7-11, 1973
3. KARDON M, PETERSON DF, BISHOP VS: Beat-to-beat regulation of heart rate by afferent stimulation of the aortic nerve. Am J Physiol **227**:598-600, 1974
4. DOUGLAS WW, RITCHIE JM, SCHAUMANN W: Depressor reflexes from medullated and non-medullated fibers in the rabbit aortic nerve. J Physiol (Lond) **132**:187-198, 1956
5. NONIDEZ JF: Aortic (depressor) nerve and its associated epithelioid body, the glomus aorticum. Am J Anat **57**:259-302, 1935
6. COLERIDGE HM, COLERIDGE JCG, DANGEL A, KIDD C, LUCK JC, SLEIGHT P: Impulses in slowly conducting vagal fibers from afferent endings in the veins, atria, and arteries of dogs and cats. Circ Res **33**:87-97, 1973
7. ALLISON JL, SAGAWA K, KUMADA M: An open-loop analysis of the aortic arch barostatic reflex. Am J Physiol **217**:1576-1584, 1969
8. GLICK G, COVELL JW: Relative importance of the carotid and aortic baroreceptors in the reflex control of heart rate. Am J Physiol **214**:955-961, 1968
9. HAINSWORTH R, LEDSOME JR, CARSWELL F: Reflex responses from aortic baroreceptors. Am J Physiol **218**:423-429, 1970
10. WARNER HR, RUSSEL RO: Effects of combined sympathetic and vagal stimulation on heart rate in the dog. Circ Res **24**:567-573, 1969
11. OBERG PA, SJOSTRAND U: Studies of blood pressure regulation: III. Dynamics of arterial blood pressure on carotid sinus nerve stimulation. Acta Physiol Scand **81**:96-109, 1971
12. JONZON A, OBERG PA, SEDIN G, SJOSTRAND U: High frequency low tidal volume positive pressure ventilation (abstr). Acta Physiol Scand **80**:21A, 1970
13. MANFREDI M: Differential block of conduction of larger fibers in peripheral nerve by direct current. Arch Ital Biol **108**:52-71, 1970
14. CARRIER GO, BISHOP VS: Interaction of acetylcholine and norepinephrine on heart rate. J Pharmacol Exp Ther **180**:31-37, 1972
15. KUMADA M, NAKAJIMA H: Field potentials evoked in the rabbit brainstem by stimulation of the aortic nerve. Am J Physiol **223**:575-582, 1972
16. PLANCHE D, ROHNER JJ, COMTE DE VAULX G: Projections corticales du nerf aortique de Cyon-Ludwig. J Physiol (Paris) **66**:211-218, 1973
17. KUNZE DL: Reflex discharge patterns of cardiac vagal efferent fibers. J Physiol (Lond) **22**:1-15, 1972
18. EVANS DHL, MURRAY JG: Histological and functional studies on the fiber composition of the vagus nerve of the rabbit. J Anat **88**:320-337, 1954
19. MALLIANI A, SCHWARTZ PJ, ZANCHETTI A: A sympathetic reflex elicited by experimental coronary occlusion. Am J Physiol **217**:703-709, 1969
20. MALLIANI A, PARKS M, TUCKETT RP, BROWN AM: Reflex increases in heart rate elicited by stimulation of afferent cardiac sympathetic nerve fibers in the cat. Circ Res **32**:9-14, 1973
21. ANGELL-JAMES JE: Effects of altering mean pressure, pulse pressure and pulse frequency on the impulse activity in fibers from the aortic arch and right subclavian artery in the rabbit. J Physiol (Lond) **214**:65-88, 1971

Cardiovascular Changes During and Following 1-Min Exposure to $+G_Z$ Stress

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PETERSON, D. F., V. S. BISHOP, and H. H. ERICKSON. *Cardiovascular changes during and following 1-min. exposure to $+G_Z$ stress.* Aviat. Space Environ. Med. 46(6):775-779, 1975.

Magnitude and duration of cardiovascular responses following $+G_Z$ forces of 1-5 G were studied in chronically instrumented anesthetized dogs. During lower G forces (+1 to $+3G_Z$), responses were variable. In most dogs during higher G forces (+4 or +5 G_Z), aortic pressure, cardiac output, left ventricular pressure, and dp/dt were all dramatically compromised. These changes were observed whether the onset of the gravitational inertial force was slow (0.1 G/s) or rapid (1.0 G/s). Cardiovascular changes after acceleration were consistent. Left atrial pressure and arterial pressure rose and a transient rise in dp/dt was often observed. Cardiac output rose briefly, then fell; hence, peripheral resistance increased. Magitude and duration of these changes were directly related to G forces during acceleration. Our results confirm that $+G_Z$ stress produces major cardiovascular changes. Our experiments also demonstrate that responses following $+G_Z$ stress may be dramatic and prolonged. Increased peripheral resistance elevates perfusion pressure and, concurrently, the increased preload may cause acute cardiopulmonary congestion.

EARLY experimental work confirmed that the limiting factor in $+G_Z$ stress tolerance was maintenance of perfusion pressure to vital organs, i.e., the central nervous system (11). Since that time, studies of acceleration in the $+G_z$ position have primarily been limited to blood pressure, heart rate, and electrocardiographic changes during acceleration (3,4,5). In conscious man, heart rate usually increases, blood pressure falls and then recovers toward control (13). ECG abnormalities may occur but do not usually persist long after cessation of acceleration (13,16,17). Similar results have been observed in experimental animals (1,2). Recently, more sophisticated methods have been used to study cardiac performance during acceleration in anesthetized experimental animals (1,6). Left ventricular pressure falls progressively further during increasing $+G_z$ forces while dp/dt responses are variable (1). Distribution of flow is not equal during acceleration, since coronary flow increases at low G forces but decreases at higher G forces (6). A redistribution of blood flow has also been observed during $+G_z$ (18,19).

Further information regarding responses associated with $+G_z$ forces is becoming increasingly necessary. Aircraft capable of maintaining high $+G_z$ levels have already been developed and future aircraft will further expand the potential G stress on pilots. Since the cardiovascular system is especially vulnerable to gravitational changes, circulatory responses to such stress should be carefully evaluated.

The purpose of this study is to examine in detail cardiovascular responses during and following $+G_z$ stress in dogs. The results reinforce the necessity for compensatory measures in order to insure adequate pilot performance and safety in advanced aircraft and suggest that factors in addition to blackout may be a source of risk to the pilot.

MATERIALS AND METHODS

Fourteen mongrel dogs (10-20 kg) were chronically instrumented under sterile surgical conditions using halothane anesthesia. A left thoracotomy through the fifth intercostal space exposed the heart and great vessels. The pericardium was opened and, through a stab incision, a solid state pressure transducer (Model P18, Konigsburg Instruments) was placed on the endocardial surface of the left ventricle for measurement of left ventricular pressure. An electromagnetic flow probe (Zepeda Instruments) was placed around the ascending aorta for measurement of cardiac output and an 18-gauge polyvinyl catheter was placed in the left atrium for measurement of pressure. In some dogs, ECG electrodes were sutured inside the chest. Lead wires for the implanted instrumentation were exteriorized at the back of the neck. Two weeks or longer were allowed for recovery. During this time, the health of each animal was monitored daily.

Prior to experimentation, animals were anesthetized

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for Laboratory Animals" prepared by the Institute of Laboratory Animal Resources—National Research Council. The research reported in this paper was conducted by personnel of The University of Texas Health Science Center at San Antonio, and of the Environmental Sciences Division, USAF School of Aerospace Medicine.

with alphachloralose. A precalibrated, solid-state pressure catheter (No. 5F, Model PC-350, Millar Instruments) was then passed retrograde via the femoral artery to the left ventricle in order to calibrate the left ventricular pressure transducer already in place. After calibration, the catheter was withdrawn to lie in the root of the aorta. Each dog was restrained on its back (+G_x) in a fiberglass animal couch which was bolted to the animal end of the USAFSAM centrifuge. The length of the arm for animal experiments is 4 m. They were positioned to receive +G_z inertial forces as the centrifuge rotated. Once the animal was properly positioned, a 15-30 min pretest period was allowed for establishment of resting levels of recorded parameters.

Animals were exposed to gravitoinertial forces of +1 to +5 G_Z consisting of either rapid (1.0 G/s) or slow (0.1 G/s) onset-to-peak G. Peak G was maintained for 60 s followed by deceleration to control. Eight animals were subjected to both slow and rapid onset-to-peak G while six of the animals underwent rapid onset only. Each animal was initially subjected to +1 or +2 G_z stress, followed by stepwise (1 G) increases after complete recovery (5-15 min) from each previous trial.

Responses were recorded on a Mark 200 Brush strip chart recorder and simultaneously on a Model 4742 Sangamo magnetic tape recorder for later analysis on an EAI 680 Analog computer.

Total peripheral resistance was calculated by the computer as mean aortic root pressure (AP) minus left ventricular end diastolic (LVEDP) divided by mean aortic flow (AF), that is:

$$TPR = \frac{AP\ (mm\ Hg) - LVEDP\ (mm\ Hg)}{AF\ (ml/min)}$$

In some cases, calibration of mean aortic flow was not possible for technical reasons; hence, our results are all expressed in percent changes in total peripheral resistance. This permitted us to include results in which mean flow values were not expressed in absolute units.

RESULTS

Responses during acceleration: Fig. 1 represents typical responses to +3 G_z stress. It is clear that during acceleration all parameters were seriously compromised. Venous return to the heart fell immediately, as indicated by a fall in left ventricular end diastolic pressure (LVEDP). Aortic root pressure (AP), left ventricular pressure (LVP), LVP dp/dt and aortic flow (AF) fell simultaneously and then usually began to recover toward control prior to the end of the acceleration period (Fig. 1). In some animals, a period of cardiac arrest was observed. One dog experienced cardiac arrest for 18 s at +4 G_z. The cardiovascular system was less dramatically affected at low G_z stress than at higher levels. The magnitude of the change in aortic arch blood pressure differed between animals but was always directly related to the magnitude of the acceleration. Eleven of the dogs always exhibited a fall in aortic pressure, while three demonstrated consistent rises in arterial pressure at most G forces tested. Average response at +1 or +2 G_z was an initial fall in pressure

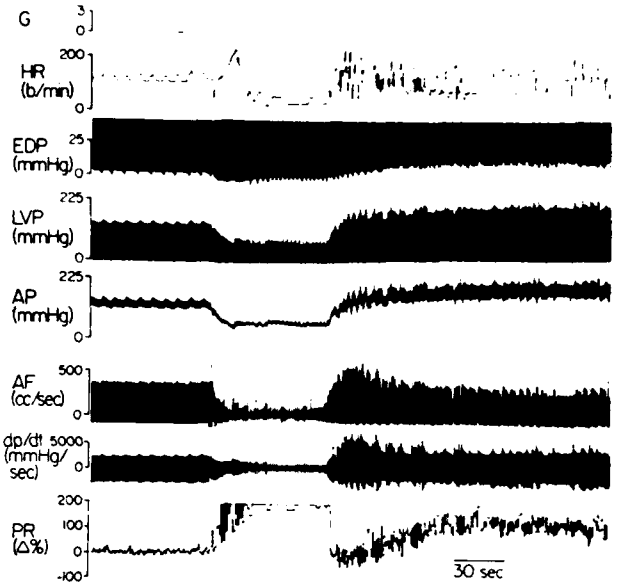

Fig. 1. Responses to +3 G_Z stress. The top trace represents the acceleration profile; HR, heart rate; EDP, end distolic pressure; LVP, left ventricular pressure; AP, aortic arch pressure; AF, pulsatile aortic flow; dp/dt, the derivative of left ventricular pressure; PR, peripheral resistance measured as percent change from control.

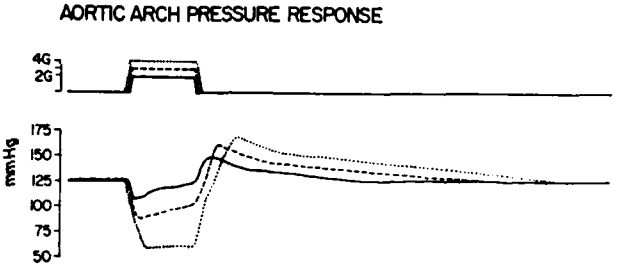

Fig. 2. Composite aortic arch pressure during and after +G_Z stress, rapid onset. Each trace represents averages for eight dogs. Curves were plotted from average values selected every 15 s and at the points of peak change. Time at peak acceleration equals 1 min.

followed by a gradual recovery toward control (Fig. 2). At +3 G_z, the average initial fall was greater and recovery toward control less complete. At +4 G_z, the initial blood pressure fall was severe in most dogs, and only a slight tendency toward recovery was evidenced in a few dogs during acceleration (Fig. 2). Eight dogs were subjected to +5 G_z based on their ability to tolerate +4 G_z stress. As seen in Table I, three dogs consistently did recover and overshoot their control aortic pressure level during acceleration. There was a tendency for compensation during slow onset (0.1 G/s) to be better than during rapid onset (1.0 G/s) in preventing blood pressure fall (Table I). The difference was significant at +4 G ($p<0.05$) which suggests that reflex compensatory mechanisms are more effective during slow onset.

TABLE I. AVERAGE MAXIMUM BLOOD PRESSURE CHANGE DURING ACCELERATION

	A				B				C			
	All Animals Included				Animals Which Compensated Poorly				Animals Which Compensated Well			
	Fast Onset		Slow Onset		Fast Onset		Slow Onset		Fast Onset		Slow Onset	
	AP	# Dogs	AP	# Dogs	AP	# Dogs	AP	# Dogs	AP	# Dogs	AP	# Dogs
1 G_Z	−15	5			−23	4			+15	1		
2 G_Z	−23	14	−6	8	−36	11	−20	6	+22	3	+30	2
3 G_Z	−42	14	−33	8	−74	11	−60	6	+23	3	+35	2
4 G_Z	−63	12*	−38	8	−97	9	−66	6	+23	3	+35	2
5 G_Z	−32**	4	−85	6	−94	2	−111	4	+28	2	−13	2

*Two dogs were not subjected to +4 G_z because of their slow recovery from +3 G_z.
**This value is biased by the two dogs in column C which always compensated for +G_z stress.
Maximum blood pressure changes in mm Hg during acceleration. Average values for all animals are given in group A. Average values for animals which always experienced a fall in aortic arch pressure are presented in group B. Averages for those dogs which usually exhibited a rise in blood pressure during acceleration are seen in group C. Note that in group B progressively lower blood pressure is observed to accompany each increase in acceleration. On the contrary, with only one exception, group C dogs produced similar blood pressure increases at each acceleration level.

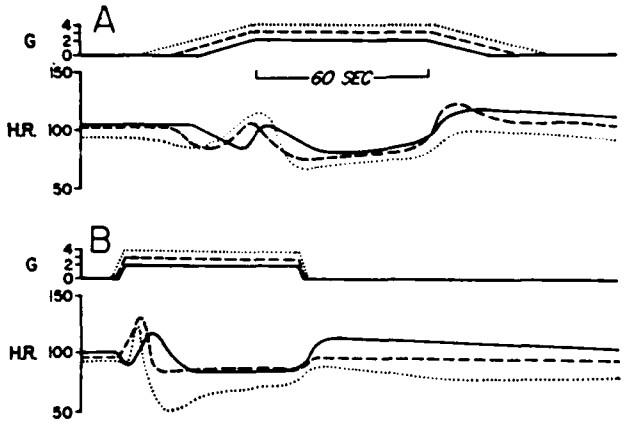

Fig. 3. Heart rate changes to +G_Z stress, fast and slow onset. Each trace represents averages for the same eight dogs.

TABLE II. MAGNITUDE OF RESPONSE AND TIME IN RECOVERY PHASE

	MLAP (4.9 mm Hg)		MAP (4.24 mm Hg)		MAP-MLAP
Slow Onset N	Δ mm Hg	return (min)	Δ mm Hg	Time to return (min)	Δ mm Hg
2 G (8)	7.0	3.8	35	4.3	28
3 G (8)	9.5	4.2	36	5.3	26
4 G (8)	12.4	5.1	41	5.6	29
5 G (6)	14.6	6.4	44	6.3	29
Fast Onset					
2 G (8)	6.0	2.5	23	2.7	17
3 G (8)	8.9	3.6	34	4.4	25
4 G (8)	13.2	5.1	43	5.9	30

Magnitude of mean left atrial pressure (MLAP) and mean arterial pressure (MAP) overshoot responses and time to return to control in the postacceleration period. Control pressures are in parentheses. Each value represents an average of the same eight animals except 5 G slow which included only 6 of the 8 dogs.

Heart rate responses were variable. Most animals displayed tachycardia soon after the onset of acceleration followed by bradycardia when onset to acceleration was rapid (Fig. 3). Average maximum heart rate change at +3 G was 8.4 s after onset. Some animals, however, responded with only one or the other response. The tendency toward bradycardia was more pronounced at higher G levels. Animals which maintained blood pressure well also tended to display either sustained tachycardia or, at least, a less than average fall in heart rate during acceleration. Heart rate responses associated with rapid onset of acceleration were much more predictable than with slow onset. When onset was slow, bradycardia usually preceded the tachycardia but, after peak G was attained, the pattern of heart rate changes was similar whether onset was slow or rapid.

Left ventricular pressure (LVP) responses were similar to arterial pressure changes. LVP reached a minimum in most trials shortly after onset of acceleration and either remained low or slowly climbed toward control during the remainder of acceleration. Maximum depression of dp/dt was directly related to the magnitude of acceleration. Cardiac output responded in much the same manner. The sudden fall in filling pressure was undoubtedly responsible for these early responses since left ventricular end diastolic pressure fell abruptly and then recovered somewhat. The fluid-filled catheter used to record left atrial pressure made accurate measurement difficult during acceleration due to movement of the heart in relation to location of the transducer; thus, left atrial pressure could not be studied directly during this period. However, in one animal a Millar catheter was passed into the right atrium in order to estimate central venous pressure. In this case, mean right atrial pressure was quantitatively similar to left ventricular end diastolic pressure.

Calculated peripheral resistance began to rise an average of 5.8 s after the onset of +G_z stress and

reached a maximum value at 20.4 s. Ordinarily it remained high throughout the 1 min of acceleration (Fig. 1). Frequently, either extremely low cardiac output or long periods of asystole made computer calculation of peripheral resistance impossible during +3 G_z, +4 G_z, and +5 G_z (Fig. 1). Average maximum measurable changes at each G_z level included: 1 G, 40%; 2 G, 82%; 3 G, 96%; 4 G, 92%; 5 G, 129%.

Postacceleration responses: After cessation of acceleration, if aortic arch pressure had fallen it always returned rapidly toward control, continued to rise higher than control, and then slowly returned back to control (Fig. 2). This "overshoot" was progressively greater at higher G levels (Table II). After +2 G_z (rapid onset), the average overshoot reached 23 mm Hg above control and required 2.7 min to return to control. After +3 G_z (rapid onset), the average overshoot reached +34 mm Hg above control and required 4.4 min to return to control. Values for +4 G_z were: 43 mm Hg (overshoot) and 5.9 min (return to control). Responses were similar whether onset of acceleration was slow or fast (Table II), and time to return to control tended to be longer after slow onset though this difference was not statistically significant.

Qualitatively, changes in left atrial pressure were similar to changes in aortic pressure. Upon cessation of acceleration, left atrial pressure immediately rose and remained high for a prolonged period of time (Table II). This overshoot, again, was greater after acceleration at higher G forces. There were no significant differences between slow and fast onset trials.

Cardiac output, after cessation of acceleration, rose transiently and reached a peak at approximately 14 s after onset of deceleration. It then fell below control levels and, finally, slowly returned to control. Peripheral resistance was calculated continuously with the analog computer from tape recorded records. Analog data provided by the computer are presented at the bottom of Fig. 1. Average peripheral resistance was considerably higher in the immediate postacceleration period.

Typically, calculated peripheral resistance fell transiently as aortic flow increased after acceleration ceased. When flow again began to fall, resistance rose. Average maximum calculated changes from preacceleration controls were: 1 G, 17%, 2 G, 19%; 3 G, 39%; 4 G, 58%; 5 G, 54%. Return to control was slow, and both flow and resistance paralleled the return of aortic root pressure. The above values do not include data from one dog which was extremely atypical. This dog experienced no change in postacceleration peripheral resistance at +1 G_z and +2 G_z. At +3 G_z, resistance rose 233% and he was not subjected to further trials.

In 67% of the animals studied at +3 G_z, an immediate transient overshoot in dp/dt max was observed when acceleration ceased. Average time to highest dp/dt max at +3 G_z was 19 s, usually followed by rapid return toward control (Fig. 1). The rapid overshoot was not observed in 33% of the animals. Either gradual return to control or sustained overshoot was observed. Time to return to control was highly variable ranging from 0.20-6.50 min.

DISCUSSION

This study confirms that, without the availability of either the M-1 maneuver or a G suit, the anesthetized dog may experience severe cardiovascular stress during +G_z (6). In addition, the unprotected animal may also experience a potentially dangerous period of cardiopulmonary congestion following +G_z stress. As a result of the elevated peripheral resistance, which undoubtedly is initiated during acceleration by both baroreflex and Cushing reflex efforts to maintain central arterial pressure (9), two undesirable responses occur in the postacceleration period: a) the elimination of excess gravitational forces causes an immediate overshoot in venous return to the heart increasing the preload and simultaneously, b) the increased peripheral resistance increases the arterial pressure, causing an elevated afterload at a time when cardiac output is attempting to recover. The lengthy time required for return to control of both left atrial and aortic pressures suggests that responses to +G_z stress are slow to recover, whether they be due to direct effects or are reflexly initiated responses. The fact that the postacceleration left atrial pressure rise was directly related to the magnitude of +G_z stress indicates that peripheral pooling was a direct result of the degree of stress in spite of simultaneous increases in peripheral vasoconstriction. The tendency for return to control to be slower after slow onset trials seems likely related to longer total duration of acceleration.

In the present study, peripheral resistance begins to increase an average of 5.8 s after the onset of +G_z, which is in agreement with previous suggestions concerning the role of the baroreceptor reflex during acceleration stress (12). The accompanying tachycardia, which had a faster onset than the increase in P.R., had little or no effect on maintaining cardiac output since flow was limited by reduction in venous return. The transient nature of the tachycardia was most likely related to the ischemia-produced bradyarrhythmia accompanying the reduced coronary perfusion (10).

Green and Miller (8) have proposed a model to describe the response of the circulatory system to acceleration stress. Though the blood pressure response they used was similar to our observations, they did not relate it to changes in cardiac output and peripheral resistance. They concluded that during acceleration the decrease in venous compliance is a major factor responsible for the return of arterial pressure toward control. Experimental evidence for a decrease in venous compliance supports their views (12,14). However, changes in venous compliance alone cannot explain our observations during the immediate post-G_z recovery period. During this period, arterial pressure progressively increased yet cardiac output, though slightly altered, remained depressed. Consequently, peripheral resistance rose steadily (Fig. 1) indicating arterial pressure recovery was dependent, in large measure, upon increasing peripheral resistance. Since increases in arterial pressure in the ranges noted in present experiments are known to have significant effects on stroke volume and MLAP in the normal animal (9), it is likely during the recovery period, when the heart is depressed, that the cardiac output and

MLAP response are significantly influenced by the increase in arterial pressure.

The observed transient increases in aortic flow and dp/dt max within 20 s post-G, which are usually accompanied by a fall in calculated total peripheral resistance, suggest a transient increase in the capacitance of resistance vessels possibly accompanied by increased vigor of contractility. A change in arterial capacitance is likely to occur since +G_z stress is known to displace visceral organs causing distortion of vessels (18).

Cineradiographic studies have shown that vessels central to the heart are elongated and reduced in diameter as much as 50% during acceleration (15). This stretch of the carotid arteries at the onset of acceleration complicates interpretation of heart rate responses. We presume that this initial stretch would stimulate carotid sinus stretch receptors and produce bradycardia (7). Since a transient initial bradycardia is often seen, especially during slow onset (Fig. 3) and never extended past the time at which peak acceleration is reached, this initial mechanical displacement is likely responsible for the bradycardia observed. As observed by others (13,16), during the deceleration period and shortly thereafter, arrhythmias are common; neural reflex regulation of heart rate is probably minimal at this time. The most common observation was a general tendency toward return to control rate with no consistent evidence of baroreceptor influence. The tendency toward postacceleration bradycardia at high G levels suggests the influence of general cardiac depression. Nevertheless, all animals were conscious and alert 1 d following trials and showed no signs of functional physiological abnormalities.

Our results have demonstrated responses to +G_z stress without the benefit of either the M-1 manuever or a G suit. Also, it is clear that the arterial baroreceptor system contributes to the overall response, although the absolute significance is not clear. Further studies will be necessary to define the quantitative effects of the anti-G suit and role of the baroreceptor system.

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REFERENCES

1. Burns, J.W. 1974. Influence of ethanol on cardiovascular tolerance to +G_Z acceleration. *Aerospace Med.* 45:19-28.
2. Burton, R.R. 1973. Positive (+G_Z) acceleration tolerances of the miniature swine: Application as a human analog. *Aerospace Med.* 44:294-298.
3. Chimoskey, J.E. 1970. Coronary blood flow and electrocardiogram during headward acceleration in unanesthetized dogs. *Aerospace Med.* 41:1028-1030.
4. Cohen, G.H., and W.K. Brown. 1969. Changes in ECG contour during prolonged +G_Z acceleration. *Aerospace Med.* 40:874-879.
5. Cohen, G.H., and W.K. Brown. 1969. Electrocardiographic changes during positive acceleration. *J. Appl. Physiol.* 27: 858-862.
6. Erickson, H.H., H. Sandler, H.L. Stone, and S. Young. 1973. Cardiac function during +G_Z acceleration. *Preprints*, Annual Scientific Meeting, Aerospace Medical Assoc., pp. 192-193.
7. Folkow, B., and E. Neil. 1971. *Circulation*, New York, Oxford.
8. Green, J.F., and N.C. Miller. 1973. A model describing the response of the circulatory system to acceleration stress. *Ann. Biomed. Engr.*, pp. 455-467.
9. Guyton, A.C. 1971. *Medical Physiology*, 4th Ed., Philadelphia, Saunders.
10. Hurst, J.W., and R.B. Logue. 1970. *The Heart.* New York, McGraw-Hill.
11. Lambert, E.H., and E.H. Wood. 1946. The problem of blackout and unconsciousness in aviators. *Med. Clin. N. Amer.* 30:833-844.
12. Newberry, P.D., and A.C. Bryan. 1967. Effect on venous compliance and peripheral vascular resistance of headward (+G_Z) acceleration. *J. Appl. Physiol.* 23:150-156.
13. Parkhurst, M.J., S.D. Leverett, Jr., and S.J. Shubrooks, Jr. 1972. Human tolerance to high, sustained +G_Z acceleration. *Aerospace Med.* 43:708-712.
14. Salzman, E.W., and S.D. Leverett, Jr. 1956. Peripheral venoconstriction during acceleration and orthostasis. *Circ. Res.* 4:450-545.
15. Sandler, H., J.E. Greenlead, B.D. Newsom, and S. Rositano. 1974. Lower-body negative pressure (LBNP) as a predictor for +G_Z tolerance after bed rest (BR). *Preprints*, Annual Scientific Meeting, Aerospace Medical Assoc., pp. 171-172.
16. Shubrooks, S.J., Jr. 1972. Changes in cardiac rhythm during sustained high levels of positive (+G_Z) acceleration. *Aerospace Med.* 43:1200-1206.
17. Shubrooks, S.J., Jr. 1973. Positive-pressure breathing as a protective technique during +G_Z acceleration. *J. Appl. Physiol.* 35:294-298.
18. Stone, H.L., H.H. Erickson, and H. Sandler. 1974. Changes in mesenteric, renal, and aortic flows with +G_X acceleration *Aerospace Med.* 45:498-504.
19. Stone, H.L., H.F. Stegall, M.B. Kardon, H. Sandler, and R.M. Payne. 1971. Changes in aortic, coronary, and carotid flows during +G_X acceleration. *J. Appl. Physiol.* 30:21-26.

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Pathways regulating cardiovascular changes during volume loading in awake dogs

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BISHOP, VERNON S., AND D. FRED PETERSON. *Pathways regulating cardiovascular changes during volume loading in awake dogs*. Am. J. Physiol. 231(3): 854-859. 1976.—The role played by the cardiac sympathetic nerves and arterial baroreceptors in the cardiovascular responses to acute volume loading was studied in conscious dogs. In 15 normally innervated animals, mean arterial pressure rose 10 mmHg, heart rate increased 38 beats/min and cardiac output 1,696 ml/min, while peripheral resistance decreased 0.99 PRU. Neither bilateral baroreceptor denervation, dorsal root sections (T_1-T_5) or surgical interruption of the left ansa subclavia altered the above responses to acute volume loading. Bilateral section of the ansa subclavia (total cardiac sympathectomy) significantly reduced the heart rate response from 35 ± 5 to 20 ± 5 beats/min but did not alter other changes. A similar reduction in heart rate response was observed following selective section of the right ansa subclavia. Intravenous infusions of epinephrine augmented the heart rate response in both normally innervated and cardiac sympathectomized dogs. It is suggested that although the primary efferent pathway for the reflex trachycardia is via the vagus, responses are modulated by sympathetic neural activity. Additionally, the nervous system was not shown to play a measureable role in the observed peripheral resistance changes.

vagus; cardiac sympathetic nerves; peripheral resistance; baroreceptors; heart rate

RECENTLY, MANY INVESTIGATIONS have centered on the role of low-pressure mechanoreceptors in or near the heart, which have been implicated in reflex regulation of cardiovascular function. Both acute volume loading (1, 3, 5, 10) and localized distension of the atrial-venous *junctions* (4, 6, 7, 11, 14) have served as models for studying these receptors. Unfortunately, results have been inconsistent when comparing either models or results of experiments by different investigators using the same model. For example, the reflex pathway for the tachycardia produced by local stretch has been reported by some to involve vagal afferent and cardiac sympathetic efferent fibers (7, 11, 14) while other investigators have reported that both vagal afferents and efferents are involved (6). In the conscious dog, the tachycardia observed with volume loading is not eliminated by beta-adrenergic blockade, thus implicating the vagus as a contributing efferent pathway (10). Most investigators indicate that the afferent pathway is in the vagus (6, 11, 13, 18), although a recent study suggests that afferents in the spinal cord might contribute to the response (8).

In conscious dogs, volume loading increases cardiac output more than it increases arterial pressure, thus peripheral resistance decreases. Vatner et al. (28) recently reported that the baroreflex control of heart rate, as judged by a pressure load, is attenuated after large-volume infusions. However, from that study it was unclear whether the baroreceptors exerted any influence on either the heart rate or peripheral resistance response to volume loading. After baroreceptor denervation, the blood pressure change during volume loading was significantly reduced by both moderate and large volumes, whereas the heart rate response was attenuated at the larger volume loads. In contrast, in anesthetized dogs, increases in cardiac output after baroreceptor denervation resulted in increased peripheral resistance (17).

This study extends previously reported responses to volume infusion. Precise afferent and efferent pathways of the reflex changes in heart rate associated with volume infusion in the conscious dog have been identified and evidence for sympathetic-parasympathetic interaction presented. In addition, effects on peripheral vascular resistance have been investigated. The possible interaction of arterial baroreceptors on both the heart rate and peripheral vascular changes have been studied by total sinoaortic baroreceptor denervation.

METHODS

Mongrel dogs were chronically instrumented under sterile surgical conditions using halothane gas. A left thoracotomy was performed through the fourth intercostal space. An 18-gauge polyvinyl catheter was placed in the left atrium through the auricular appendage. A calibrated electromagnetic flow probe was affixed around the ascending aorta in 15 animals. The calibration of all flow probes were checked as previously described (1). A piece of surgical suture was looped around both left ansa subclavia for later cardiac sympathectomy (23). An 18-gauge polyvinyl catheter was inserted into either the internal mammary artery or in the carotid artery through a cervical incision. At the time of thoracotomy, or a few days later, a 10-gauge polyvinyl catheter was inserted into the superior vena cava through the left jugular vein. Approximately 2 wk were allowed for recovery, during which time the health of the animal was monitored daily. At the time when experiments were performed, body temperature and electrocardiogram (ECG) were normal.

Experimental protocol. ECG, heart rate, systemic blood pressure, and left atrial pressure were recorded on a Beckman paper oscillograph using the required transducers, couplers, and amplifiers. Tyrode's solution warmed to 37.5°C was infused through the large catheter in the jugular vein using a Holter pump (model RE161). The rate of the infusion was controlled to produce a steady rise in left atrial pressure. Infusions were normally performed over a 1- to 4-min period until the heart rate had reached a constant level, which was not exceeded despite further rise in left atrial pressure.

The total volume of Tyrode's solution required to reach a maximum heart rate ranged between 300 and 800 ml (averaged 400 ml) (1). The hemodilution effect ranged from −2 to −5%. In previous studies, identical cardiac responses were observed with intravenous infusions of blood or Tyrode's solution (2). Infusions were performed in all animals under control conditions. Additional infusions were performed after each surgical denervation described below and in some cases during a constant intravenous infusion of epinephrine. The above parameters were continuously recorded during the infusions. All experiments were performed while the animals rested unrestrained in a hammock. Two days separated each infusion.

Total peripheral resistance, expressed in peripheral resistance units (PRU), was calculated

$$\text{PRU} = \frac{\text{mean arterial blood pressure (mmHg)} - \text{central venous pressure (mmHg)}}{\text{mean aortic flow (ml/min)}} \times 60 \text{ s/min}$$

Values are reported as means or mean differences ± standard errors (SE). Statistical evaluation was made by use of the Student t test for paired comparisons (26). $P < 0.05$ was considered significant.

Cardiac sympathectomy. In six dogs a sterile thoracotomy was performed a 2nd time in order to loop surgical suture around the right ansa subclavia distal to the right stellate ganglion. After establishing the control responses to acute volume loading this suture, as well as the one previously placed around the left ansa subclavia, was pulled to selectively cut the sympathetic nerves to the heart (20). The sympathectomy was performed when the animal was anesthetized. After recovery the animals were reentered into the experimental protocol. In three additional animals with only catheters implanted, the right ansa subclavia was looped and the response to acute volume loading was observed before and after the removal of the right ansa subclavia while sympathetic innervation to the left side remained intact.

Baroreceptor denervation. After control responses were obtained, five animals were anesthetized with sodium pentothal (30 mg/kg) and a midcervical incision was made. The carotid sinuses were located and all the vessels and other tissue above the bifurcation of the common carotid arteries isolated, ligated, and sectioned except the external and internal carotid arteries. All remaining excess tissue was stripped from the vessels originating from the common carotid arteries. These procedures eliminated the reflex heart rate and blood pressure responses to carotid occlusion (22, 23). All branches of the vagus within 2 cm below the superior laryngeal nerves were also sectioned. Presumably this procedure denervated the aortic arch and combined with carotid sinus denervation resulted in an immediate rise in blood pressure. Heart rate responses to the injection of phenylephrine (10 μg) were abolished or drastically reduced. These animals were allowed several days to recover before reentering the experimental protocol.

Sympathetic deafferentation. In four additional animals the heart rate response to acute volume loading was established. Subsequently, these animals were anesthetized with sodium pentothal (30 mg/kg) and under sterile conditions the dorsal roots T_1–T_5 were sectioned. The animals were allowed to recover 10–14 days and were reentered into the experimental protocol.

Drug administration. In order to artificially simulate an increase in sympathetic background activity, epinephrine was infused intravenously in four additional animals instrumented with catheters only. The infusion rates were controlled so that heart rate was only minimally increased. Subsequently, the predetermined level of epinephrine infusion was maintained during the infusion of Tyrode's solution.

RESULTS

A total of 66 control volume infusions were performed in 22 conscious dogs. In each animal the infusion rate was controlled in order to produce a continuous rise in left atrial pressure. Increases in cardiac output and heart rate typically paralleled the increase in left atrial pressure during the first 1–2 min of infusion. These responses then plateaued at levels consistent for each individual animal at which time the infusion was terminated. Mean cardiac output increased from an average of 2,554 ± 157 ml/min to an average maximum of 4,250 ± 280 ml/min (n = 15 dogs). Average heart rate changes were from 109 ± 4 to 147 ± 6 beats/min. Although cardiac output increased dramatically, the change in arterial pressure was not great (100 ± 3 to 110 ± 3 mmHg). Consequently, the peripheral vascular resistance was markedly reduced (from 2.42 ± 0.19 to 1.43 ± 0.15 PRU).

In order to identify specific efferent pathways involved in the heart rate response, the cardiac sympathetic nerves were selectively cut. In five dogs, removal of the left ansa subclavia nerves only was without effect on the heart rate response to volume infusion. However, in three animals, section of the right ansa subclavia alone reduced the ΔHR response to volume loading from 35 to 17 beats/min. In six animals, a total of 24 infusion curves were obtained before and after bilateral section of the ansa subclavia nerves. Cutting both the right and left ansa subclavia reduced the average resting HR from 110 ± 5 to 85 ± 4 beats/min ($P < 0.05$). Arterial pressure, cardiac output, and peripheral resistance were all slightly, but not significantly, reduced due to nerve section (Table 1). The peak change in heart rate due to infusion was also reduced from the average control change of 35 ± 5 to 20 ± 5 beats/min ($P < 0.05$). These

TABLE 1. *Comparison of hemodynamic responses to volume loading*

	A (n = 6)		B (n = 5)		C (n = 4)	
	Control	Total Cardiac Sympathectomy	Control	Baroreceptor Denervation	Control	Left Sympathectomy
Cardiac output, ml/min						
Resting	2,500 ± 229	2,412 ± 197	2,598 ± 169	2,608 ± 201	2,583 ± 230	2,458 ± 318
Peak	4,446 ± 309	3,396 ± 254	4,040 ± 490	3,987 ± 367	4,218 ± 691	3,831 ± 711
$\bar{d}$	1,946 ± 207	983 ± 168	1,442 ± 343	1,377 ± 193	1,635 ± 481	1,370 ± 438
Heart rate, beats/min						
Resting	110 ± 5	85 ± 4	105 ± 6	102 ± 3	112 ± 9	106 ± 7
Peak	146 ± 7	105 ± 6	142 ± 8	131 ± 4	152 ± 15	146 ± 17
$\bar{d}$	35 ± 5	20 ± 5	35 ± 4	33 ± 6	39 ± 10	40 ± 12
Mean arterial pressure, mmHg						
Resting	97 ± 6	88 ± 5	102 ± 5	107 ± 5	103 ± 6	99 ± 2
Peak	109 ± 4	97 ± 5	112 ± 3	114 ± 5	110 ± 7	111 ± 2
$\bar{d}$	12 ± 3	9 ± 4	10 ± 3	8 ± 3	6 ± 6	11 ± 2
Stroke volume, ml/beat						
Resting	22.8 ± 2.1	28.8 ± 3.0	25 ± 1.64	25.7 ± 1.87	22.5 ± 1.5	22.7 ± 3.1
Peak	31.1 ± 3.8	32.4 ± 2.8	27.4 ± 2.19	28.3 ± 3.8	26.6 ± 1.5	25.7 ± 2.2
$\bar{d}$	8.8 ± 1 6	3.7 ± 1.4	2.4 ± 2.3	2.6 ± 3.1	4 ± 1.45	2.9 ± 1.1
Peripheral vascular resistance, mmHg·s ml^{-1}						
Resting	2.38 ± 0.28	2.18 ± 0.23	2.32 ± 0.20	2.41 ± 0.25	2.35 ± 0.06	2.48 ± 0.48
Peak	1.30 ± 0.12	1.49 ± 0.14	1.48 ± 0.20	1.52 ± 0.16	1.45 ± 0.26	1.67 ± 0.38
$\bar{d}$	−1.08 ± 0.17	−0.69 ± 0.16	−0.84 ± 0.08	−0.89 ± 0.12	−0.90 ± 0.22	−0.81 ± 0.15

$\bar{d}$, mean difference.

values were not different from those observed after right cardiac sympathectomy only. In addition, as shown in Fig. 1, the slope of the ascending portion of the heart rate response curve was decreased following total cardiac sympathectomy. Thus, it is apparent that the right, but not the left, cardiac sympathetic nerves contribute to the reflex HR adjustment to volume loading. Also, since the vagus remained as the only innervation to the heart, it too contributed to the heart rate response. Because the initial HR as well as the maximum HR response was reduced following section of the right ansa subclavia, the cardiac sympathetic nerves may only modulate the HR response to vagal withdrawal.

It has been demonstrated that HR responses to changes in vagal efferent activity are modulated by the existing level of sympathetic activity (16). To evaluate the possibility that the contribution of the sympathetic activity to the HR responses observed during volume loading is modulatory, intravenous infusions of epinephrine were initiated in four animals prior to the inscription of the volume infusion curve. As shown in Table 2, epinephrine significantly augmented the ΔHR response to the volume infusion (range, 10–28 beats/min increased response). After bilateral section of the ansa subclavia intravenous epinephrine also increased the ΔHR to volume loading (range 18–23 beats/min increased response).

Since selective interruption of the ansa subclavia might attenuate the heart rate response as a result of partial deafferentation, the heart rate response was examined in four additional animals before and following sectioning of the dorsal roots (T_1–T_5). This presumably deafferented nonvagal cardiac innervation (20). Neither resting heart rate (before, 95 ± 5 beats/min; after, 94 ± 8 beats/min), nor the change in heart rate (before, 43 ± 6 beats/min; after 36 ± 4 beats/min) were significantly altered due to dorsal root sectioning. Thus, it appears that the afferent pathway for the heart rate

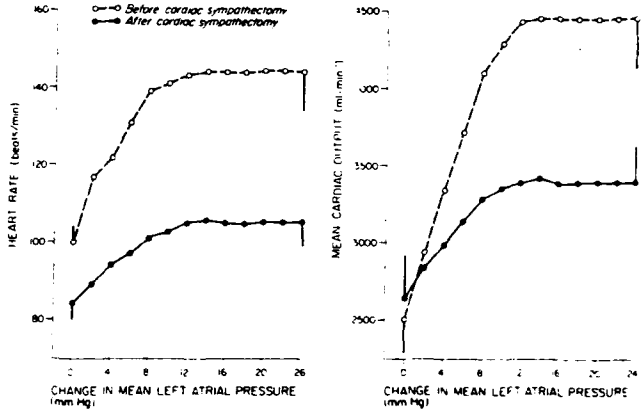

FIG. 1. Heart rate and cardiac output responses to volume loading before and after bilateral cardiac sympathectomy. Average responses from each animal were considered a single observation. Each curve represents average of same 6 animals.

response during volume loading lies primarily in the vagus nerves.

As shown in Table 1 and Fig. 1, the influence of the sympathetic nerves on the cardiac output response was related to the heart rate changes. Under normal conditions, volume loading increased the cardiac output from 2,500 ± 229 to 4,446 ± 309 ml/min. Following bilateral section of the ansa subclavia nerves, the cardiac output response was significantly reduced (control to peak was 2,412 ± 197 to 3,396 ± 254 ml/min) and was proportional to the reduction in heart rate. As a result of the lower resting heart rate, the stroke volume during control conditions was increased (from 22.8 ± 2.1 to 28.8 ± 3.0 ml/beat). However, unlike the control response, stroke volume was not significantly increased due to volume loading after bilateral section of the cardiac sympathetic nerves (Table 1). Volume-induced peripheral resistance changes were attenuated by cardiac sympa-

TABLE 2. *Influence of epinephrine on heart rate response to volume loading*

	Heart Rate, beats/min			
	C	Epi	TS	Epi + TS
Rest	89	98	89	84
Peak	129	151	107	122
d	37	53	18	38
Range	31-39	41-67	15-21	33-45

n = 4. Intravenous infusion of epinephrine (Epi) was maintained during volume loading before cardiac sympathectomy and after cardiac sympathectomy (Epi + TS). These responses are compared with control (C) infusions and infusion after cardiac sympathectomy (TS) without epinephrine. d, mean difference.

thectomy even though the final total peripheral resistance reached was not significantly different. The reduction in cardiac output may account for this change. No significant difference in the peripheral resistance response was noted after left sympathectomy alone (Table 1).

In order to identify a possible involvement of the arterial baroreceptors in the heart rate or peripheral resistance changes, sinoaortic denervations were performed in five animals from which 12 infusion curves had been obtained. Following the denervation, 12 additional infusions were performed. As shown in Table 1 and Fig. 2, neither the resting values of heart rate and cardiac output nor their responses to the infusions were significantly altered. Acute volume loading caused similar declines in peripheral resistance (from 2.35 to 1.45 PRU before baroreceptor denervation, and from 2.48 to 1.67 PRU after denervation) which appeared to be linearly related to the rise in cardiac output (Fig. 3). This suggests that mechanisms other than the arterial baroreceptors are involved in the regulation of peripheral resistance during acute volume loading.

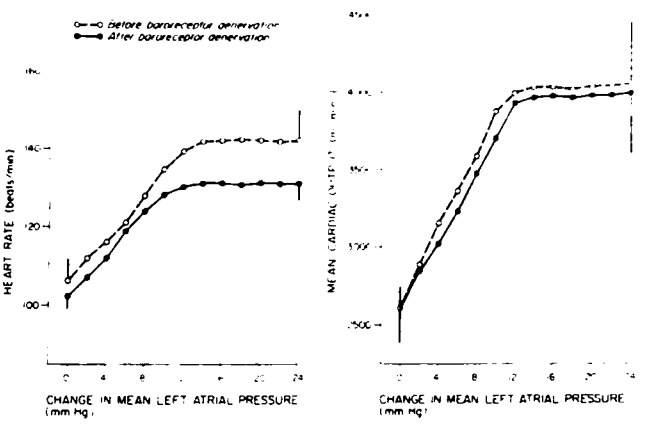

FIG. 2. Heart rate and cardiac output responses to volume loading before and after arterial baroreceptors denervation. Each curve represents average of same 5 animals.

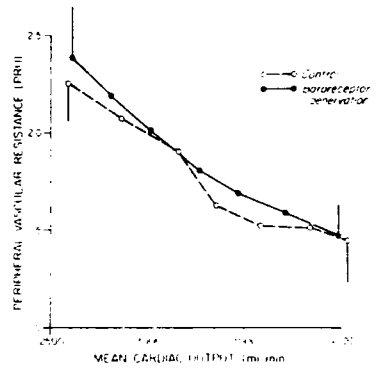

FIG. 3. Relationship between peripheral vascular resistance and cardiac output during volume infusion. Each curve represents average of same 5 dogs.

DISCUSSION

Evidence has been presented to show that increases in arterial pressure during acute volume loading are associated with a significant tachycardia which is influenced primarily by changes in efferent vagus nerve activity with a lesser but significant contribution through the right cardiac sympathetic nerves. The fact that the ΔHR response to volume loading was less after section of the right, but not the left, ansa subclavia adds additional support for the role of the right cardiac sympathetic nerves in the reflex HR response to volume loading. Furthermore, in our study, since removal of the dorsal roots from T_1 to T_5 did not alter the heart rate response to volume loading, one may assume that the afferent pathway is in the vagus nerves. It is clear from the results of this study that sino-aortic denervation did not alter the magnitude of the heart rate response. A similar observation was noted by Vatner et al. (28) during modest infusions.

Since the results in this study and others (10, 28) have demonstrated that tachycardia due to volume loading is influenced by both vagal and sympathetic efferent pathways, it is possible that the magnitude of the response may depend on a reciprocal relationship between vagal and sympathetic neural activity reaching the SA node (9, 12). Direct nerve stimulation has indicated that control of heart rate is dominated by the vagus (16, 29). When vagal efferent activity is held constant, large changes in cardiac sympathetic efferent activity are required to alter heart rate substantially (15, 16). On the other hand, from the same studies, it is apparent that when sympathetic activity is held constant, small changes in vagal nerve activity can cause large changes in heart rate especially when resting sympathetic activity is high. In our study, it is conceivable that the heart rate response could result from a combination of small decreases in vagal activity and concurrently much larger increase in sympathetic activity. However, large increases in sympathetic activity seems unlikely for the following combination of reasons: *i*) approximately 60% of the heart rate response remained following the elimination of the sympathetic input. *ii*) Following vagal blockade, the tachycardia response to volume loading is eliminated. This suggests that the sympathetic activity is not increasing appreciably (10, 28), but rather, the

vagal influences were more significant in the face of background sympathetic activity.

The concept that the background level of sympathetic activity can modify vagal influences was tested in the present study by controlled infusion of epinephrine which had only a slight effect on the resting heart rate. This caused the peak heart rate response to acute volume loading to be increased in either intact or cardiac sympathectomized animals. From this, one can conclude that the magnitude of the response to vagal withdrawal can be increased by increasing the existing sympathetic activity (i.e., concentration of catecholamine at the SA node). This conclusion is supported by the work of Levy and Zieske (16) in which combined electrical stimulation of the vagus and sympathetic nerves to the heart was employed. When the basal heart rate was moderately elevated by tonic sympathetic stimulation, increases in heart rate due to reduced vagal stimulation were much more dramatic (15, 16). Similar heart rate dependency upon the resting sympathetic activity is evidenced when comparing the effects of vagal blockade on the control heart rate before and after elimination of sympathetic influences (10, 28).

Although volume loading may stimulate receptors, in addition to those shown to be in the atrial-venous junction, similarities do exist between the responses observed during volume loading and some of those reported during direct tissue stretch. Edis et al. (6) reported that the magnitude of the heart rate response to stretch of the pulmonary vein-atrial junction depended upon both efferent vagal and cardiac sympathetic nerves. In their study, when carotid sinus pressure was manipulated to maintain heart rate lower than 140–150 beats/min stretch of the junction produced tachycardia. When manipulation of the sinus pressure forced heart rate above 150 beats/min (presumably withdrawing the vagus and activating the cardiac sympathetic nerves) stretch of the pulmonary vein-atrial junction caused bradycardia. Reversal of responses is explainable if one considers that the efferent activity in both groups of nerves could simultaneously be reduced during distension. This concept is supported by the work of Levy et al. (16) in which withdrawal of vagal activity dominates heart rate changes until the vagus is silent. Further evidence that cardiac sympathetic activity can withdraw during volume infusion is the bradycardia following vagal blockade (2, 10, 28) as well as the bradycardia seen during coronary occlusion in sinoaortic denervated animals with heart rates greater than 150 beats/min (23).

Our study also demonstrated an important vasodilator effect during volume infusion. Sinoaortic denervation failed to demonstrate high pressure receptor involvement in these peripheral resistance changes. Previous studies have shown a small vasodilator effect by stimulation of atriovenous junction receptors (6) but volume loading studies have failed to support a substantial contribution through afferent vagal involvement (3). Therefore, one may presume that the observed peripheral resistance responses primarily involved direct vasodilator influences secondary to increased cardiac output.

Acute volume loading in the conscious animal resulted in a proportionately larger increase in cardiac output than arterial pressure. Consequently, total peripheral resistance declined as cardiac output was increased. Liedtke et al. (17) changed cardiac output in anesthetized dogs by altering cardiac pump performance either through changes in coronary perfusion or calcium ion concentration in the perfusion of the coronary beds. They also observed a similar decline in peripheral resistance with increasing cardiac output. However, following bilateral baroreceptor denervation and vagotomy, they observed an increase in peripheral resistance with increasing cardiac output. They concluded that total body autoregulation occurred when the arterial baroreflexes from the carotid and aortic arch were eliminated. In our study, bilateral removal of the carotid and aortic baroreceptors did not alter the relationship between resistance and cardiac output.

Sagawa and Eisner (24) also failed to observe significant whole-body autoregulation in anesthetized vagotomized dogs either before or after the abrogation of arterial baroreflexes. Assuming a rectilinear relationship between pressure and flow, they observed a near constant peripheral resistance when pressure and flow were varied from 60 to 140%. Although much less than we observed, the pressure-flow relationship in their study was convex toward the flow axis, indicating that as cardiac output increases above normal the peripheral resistance declines. They suggested that this may be due to arterial baroreceptor reflexes. However, in the conscious animal, baroreceptor denervation did not alter the relationship, suggesting other mechanisms for vasodilitation at flow rates above normal.

A detailed study by Shepherd et al. (25) may explain our observations. They noted little autoregulation in areflexive dogs even though arterial pressure was reduced to 50%. Oxygen delivery was found to be maintained by increasing oxygen extraction. However, when initial O_2 extraction ((a-v)O_2 difference) was increased either by epinephrine infusions or ventilatory induced hypoxia, flow autoregulation occurred. In these conscious animals it is unlikely that the O_2 delivery is limited by either extraction or flow. Consequently, at above normal flow rates the pressure-flow relationship may become convex toward the flow axis.

Previous studies have demonstrated a role of the cardiopulmonary receptors in the regulation of vascular resistance (6, 19, 21). Furthermore, vagal afferents have been shown to inhibit the sympathetic outflow to the peripheral vascular beds (19). However, in conscious dogs cold block of the vagus does not apparently alter the cardiac output and arterial pressure relationship during volume loading (2). However, it is possible that volume loading decreases the restraint exerted by the arterial baroreceptors (27), thereby minimizing the influence of the low-pressure receptors on the sympathetic outflow (19).

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REFERENCES

1. BISHOP, V. S., AND L. D. HORWITZ. Effects of altered autonomic control on left ventricular function in conscious dogs. *Am. J. Physiol.* 221: 1278–1282, 1971.
2. BISHOP, V. S., H. L. STONE, AND A. C. GUYTON. Cardiac function curves in conscious dogs. *Am. J. Physiol.* 207: 677–682, 1964.
3. BISHOP, V. S., H. L. STONE, AND L. D. HORWITZ. Effects of tachycardia and ventricular filling pressure on stroke volume in the conscious dog. *Am. J. Physiol.* 220: 436–439, 1971.
4. CARSWELL, F., R. HAINSWORTH, AND J. R. LEDSOME. The effects of distention of the pulmonary vein-atrial junctions upon peripheral vascular resistance. *J. Physiol., London* 207: 1–14, 1970.
5. COLERIDGE, J. C. G., AND R. J. LINDEN. The effect of intravenous infusions upon the heart rate of the anesthetized dog. *J. Physiol., London* 128: 310–319, 1955.
6. EDIS, A. J., D. E. DONALD, AND J. T. SHEPHERD. Cardiovascular reflexes from stretch of pulmonary vein-atrial junctions in the dog. *Circulation Res.* 27: 1091–1100, 1970.
7. FURNIVAL, C. M., R. J. LINDEN, AND H. M. SNOW. Reflex effects on the heart of stimulating left atrial receptors. *J. Physiol., London* 218: 447–463, 1971.
8. GUPTA, P. D. Spinal autonomic afferents in elicitation of tachycardia in volume infusion in the dog. *Am. J. Physiol.* 229: 303–308, 1975.
9. HAKUMAKI, M. O. K. Vagal and sympathetic efferent discharge in the Bainbridge Reflex of dogs. *Acta Physiol. Scand.* 85: 414–417, 1972.
10. HORWITZ, L. D., AND V. S. BISHOP. Effect of acute volume loading on heart rate in the conscious dog. *Circulation Res.* 30: 316–321, 1972.
11. KAPPAGODA, C. T., R. J. LINDEN, AND D. A. SAUNDERS. The effect on heart rate of distending the atrial appendages in the dog. *J. Physiol., London* 225: 705–719, 1972.
12. KARDON, M. B., D. F. PETERSON, AND V. S. BISHOP. Reflex bradycardia due to aortic nerve stimulation in the rabbit. *Am. J. Physiol.* 225: 7–11, 1973.
13. KARIM, F., C. KIDD, C. M. MALPUS, AND P. E. PENNER. The effects of stimulation of the left atrial receptors on sympathetic efferent nerve activity. *J. Physiol., London* 227: 243–260, 1972.
14. LEDSOME, J. R., AND R. J. LINDEN. A reflex increase in heart rate from distention of the pulmonary-vein-atrial junctions. *J. Physiol., London* 170: 456–473, 1964.
15. LEVY, M. N. Sympathetic-parasympathetic interactions in the heart. *Circulation Res.* 29: 437–445, 1971.
16. LEVY, M. N., AND H. ZIESKE. Autonomic control of cardiac pacemaker activity and atrioventricular transmission. *J. Appl. Physiol.* 27: 465–470, 1969.
17. LIEDTKE, A. J., C. W. URSCHEL, AND E. S. KIRK. Total systemic autoregulation in the dog and its inhibition by baroreceptor reflexes. *Circulation Res.* 32: 673–677, 1973.
18. LINDEN, R. J. Function of cardiac receptors. *Circulation* 48: 463–480, 1973.
19. MANCIA, G., D. E. DONALD, AND J. T. SHEPHERD. Inhibition of adrenergic outflow to peripheral blood vessels by vagal afferents from the cardiopulmonary region in the dog. *Circulation Res.* 33: 713–721, 1973.
20. MIZERES, N. J. Origin and course of cardioacceleration fibers in the dog. *Anat. Record* 132: 261–279, 1958.
21. PELLETIER, C. L., A. J. EDIS, AND J. T. SHEPHERD. Circulatory reflex from vagal afferents in response to hemorrhage in the dog. *Circulation Res.* 29: 626–634, 1971.
22. PETERSON, D. F., AND V. S. BISHOP. Reflex blood pressure control during acute myocardial ischemia in the conscious dog. *Circulation Res.* 34: 226–232, 1974.
23. PETERSON, D. F., R. L. KASPAR, AND V. S. BISHOP. Reflex tachycardia due to temporary coronary occlusion in the conscious dog. *Circulation Res.* 32: 652–659, 1973.
24. SAGAWA, K., AND A. EISNER. Static pressure-flow relation in the total systemic vascular bed of the dog and its modification by the baroreceptor reflex. *Circulation Res.* 36: 406–413, 1975.
25. SHEPHERD, A. P., H. J. GRANGER, E. E. SMITH, AND A. C. GUYTON. Local control of tissue oxygen delivery and its contribution to the regulation of cardiac output. *Am. J. Physiol.* 225: 747–755, 1973.
26. SNEDECOR, G. W., AND W. G. COCHRAN. *Statistical Methods*. (6th ed.) Ames, Iowa: Iowa State Univ. Press, 1967, p. 91–98.
27. STINNETT, H. O., D. F. PETERSON, AND V. S. BISHOP. Cardiovascular responses to electrocardiogram-coupled stimulation of rabbit aortic nerve. *Am. J. Physiol.* 230: 1374–1378, 1976.
28. VATNER, S. F., D. H. BOETTCHER, G. R. HEYNDRICKX, AND R. J. MCRITCHIE. Reduced baroreflex sensitivity with volume loading in conscious dogs. *Circulation Res.* 37: 236–242, 1975.
29. WARNER, H. R., AND R. O. RUSSELL. Effect of combined sympathetic and vagal stimulation on heart rate in the dog. *Circulation Res.* 24: 567–573, 1969.

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Cardiovascular responses to electrocardiogram-coupled stimulation of rabbit aortic nerve

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STINNETT, H. O., D. F. PETERSON, AND V. S. BISHOP. *Cardiovascular responses to electrocardiogram-coupled stimulation of rabbit aortic nerve.* Am. J. Physiol. 230(5): 1374–1378. 1976.—Electrical stimulation of the rabbit's aortic nerve during one or more cardiac cycles resulted in a reflex fall in heart rate and mean arterial blood pressure (MAP). The onset of bradycardia and of fall in MAP were independent of the number of beats stimulated. The initial slope of the heart rate and MAP responses increased as the number of beats stimulated increased, reaching a maximum at five beats of stimulation. Bradycardia peaked 8 and 10 beats after the end of one and two cycles of stimulation, respectively, while the peak response occurred at, or prior to, the end of stimulation when 12 or more beats were involved. Onset and recovery of both responses were consistent, and seldom did MAP indicate a return toward control during stimulation. Thus, central nervous system modulation of sympathetic activity to the peripheral vasculature was sustained as long as the aortic nerve input was maintained. However, reflex control of heart rate was more complex, involving simultaneous alteration in both vagal and sympathetic efferent activity.

baroreceptor reflexes; heart rate; blood pressure

CONTINUOUS, SUPRAMAXIMAL STIMULATION of the aortic nerve results in sustained depression in heart rate (6) and blood pressure (3, 10). The chronotropic response has been shown to involve activation of both myelinated and unmyelinated afferent fibers that reflexly modify both vagal and sympathetic influences on the heart (6, 7). Blood pressure changes appear to be influenced by activation of myelinated and unmyelinated afferents (3, 10), and the efferent limb associated with this response is in the sympathetic nerves that alter peripheral vascular tone (6, 7). Recent work has indicated that electrical stimulation of the afferent aortic nerve, confined to one cardiac cycle, can produce transient bradycardia (5). Similar direct evidence for beat-to-beat blood pressure regulation has not been demonstrated. In addition, little is known about the time course of reflex blood pressure adjustments or of their temporal relationship with heart rate changes during aortic nerve activation.

The purpose of this study was to examine quantitatively the extent and time course of the reflex change in heart rate and blood pressure resulting from electrical stimulation of the left aortic nerve in the rabbit. Stimuli were synchronized with the R wave of the electrocardiogram (ECG) and the total duration of stimulation was varied in order to determine whether the responses or their relationship to each other were dependent on the number of cardiac cycles activated.

The rabbit was used in this study because its aortic nerve is easily identifiable, anatomically separate, and composed almost entirely of afferent fibers originating from baroreceptors in or near the aortic arch (1, 10).

METHODS

Fourteen rabbits weighing 1.7–2.5 kg were anesthetized with pentobarbital sodium via an ear vein (Diabutal, Diamond Laboratories, Inc., 30 mg/kg iv). Supplemental anesthetic was administered through a cannulated femoral vein. The femoral artery was also cannulated and connected to a Statham P23db strain gauge for blood pressure recordings. Heart rate was monitored via sternal needle electrodes connected to a Beckman 9857B cardiotachometer coupler. Blood pressure and heart rate were initially recorded on a Beckman R411 oscillograph with parallel output signals to a Digital Equipment Corporation PDP-8/E digital computer. A tracheotomy was performed and the animals were artificially ventilated by the technique published previously (6) to assure maintenance of normal blood Po_2, Pco_2, and pH. Through a midventral incision, the left aortic nerve (LAN) was located in the cervical region, carefully isolated from surrounding tissue for about 1 cm, sectioned near the sternum, and bathed in mineral oil, as previously described (6). The central end of the LAN was placed onto bipolar (plantinum iridium) electrodes that were connected to a Grass SD9 stimulator. Nerve stimulation was accomplished by synchronization of the Schmidt trigger of the computer with the R wave of the ECG. The Schmidt trigger then activated the stimulator. Thus, regulation of stimulus timing and stimulus parameters, as well as continuous calculation of the length of each R-R interval and beat-to-beat mean blood pressure, was accomplished with the computer and special computer program systems. An experimental trial consisted of: *1)* 10 successive control cardiac cycles, *2)* bursts of electrical stimuli coupled to each R wave of the ECG beginning with the 11th interval and continuing through a predetermined number of beats, and *3)* continuous data collection through recovery to control. Each burst of electrical stimulation was made up to 10 square-wave impulses (10 V) inserted 10 ms after the recorded R wave of the ECG. The impulse duration was 0.3 ms, stimulus frequency was 80 Hz, and burst duration was 113 ms. Each combination of stimulus condi-

tions was repeated 10 times in succession, and the average R-R interval and blood pressure were calculated by the computer for each cardiac cycle during control as well as during and after stimulation periods. The number of beats stimulated included 1, 2, 5, 12, 20, 40, and 120, and the order of stimulation was randomized between animals. From the averaged trial data, the peak change in R-R interval duration and blood pressure, as well as the latencies to onset and peak responses, were calculated. Digital analysis was made by the computer and computer-linked oscilloscope displays of the responses were obtained and photographed.

The latency to onset (LTO) was measured as the time from the beginning of stimulation to the first beat indicating a fall in heart rate or blood pressure below the average control values. The initial mean slope values for the various curve components, representing the initial rate of change in the respective response, were calculated based on the average unit change (ms or mmHg, respectively) per unit time during the first 10 beats after onset. Latency to peak (LTP) bradycardia was measured as the time from the beginning of the stimulus to the end of the beat with the longest interval time. The LTP hypotension was measured as the time from the beginning of stimulation to the end of the first beat having the minimum mean blood pressure. Time constants for the recovery after stimulation were determined by measuring the time from initiation of the recovery response until heart rate or blood pressure had recovered toward control by 67% of the original displacement.

Values are reported as the mean or mean difference ± SE. Statistical evaluation was made by use of the Student t test for paired comparisons; $P < 0.05$ was considered significant.

RESULTS

Figure 1 presents average heart rate (measured in R-R interval time) and mean blood pressure response curves recorded simultaneously during 120 beats of stimulation for one animal. During LAN stimulation of this number of heartbeats, both maximal reflex bradycardia and hypotension were attained in all animals studied. Each curve and point in the curve represents the average value for 10 successive trials. After aortic nerve stimulation began (zero time) onset of heart rate changes occurred quickly (2nd beat). Onset of mean blood pressure change, however, did not occur until the 9th or 10th beat.

The heart rate response during 120 beats of stimulation was characterized by an initial rapid fall interrupted by an abrupt decrease in slope ("shoulder") (Fig. 1). Usually, heart rate continued to fall slightly but the difference between the shoulder and the peak response did not prove to be significant. In all cases there was a slight tendency for heart rate to return toward control before stimulation ceased, probably a result of intact carotid sinus baroreceptors responding to the falling blood pressure.

A reflex arterial blood pressure response similar to that shown in Fig. 1 was obtained in all animals. Similar onsets were also observed in all animals, but variations in the latency to peak and the peak response were found (Fig. 2*B*).

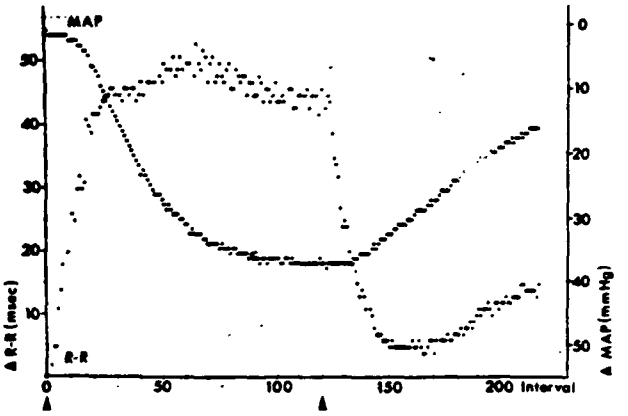

FIG. 1. Oscillograph display of reflex bradycardia (change in R-R interval time of ECG) and blood pressure fall [change in mean arterial pressure (MAP)] produced in 1 rabbit during electrical stimulation of left aortic nerve (LAN) for 120 heartbeats. Both response curves are average of 10 trails. Stimulation initiation is at zero. Each point represents average R-R or MAP value recorded per heartbeat and displayed on a Digital Equipment Corporation PDP-8 computer oscilloscope. *Ordinate:* R-R (5 ms/division left scale; bottom to top) and decrease in MAP (5 mmHg/division right scale; top to bottom). *Abscissa:* heartbeat number from beginning of stimulus (zero). Arrows indicate beginning and end of stimulation.

When stimulation of the LAN was terminated, the onset of recovery for heart rate began in two cardiac cycles, whereas mean arterial pressure changes, on the average, were delayed eight cycles.

Bradycardia occurs after supramaximal stimulation of the aortic nerve during one R-R interval. Our results confirm this and further demonstrate that a blood pressure depression also occurs (Fig. 2*B*). As shown in Fig. 2, the time course and magnitude of the heart rate and blood pressure responses were influenced by the number of beats in which the LAN was stimulated. Each curve represents the average of response for all animals subjected to that number of beats of stimulation. For clarity, results for 2 and 12 beats of stimulation were omitted from Fig. 2. The average control R-R interval was 203.5 ± 3.8 ms (SE); the range was 195.4–208.7 ms. The onset of bradycardia was essentially the same for all levels of stimulation, occurring between the first and second beat, mean 1.4 ± 0.3 beats (LTO, 235 ± 77 ms). However, the initial mean slope increased progressively as the number of beats stimulated increased from one to five (Figs. 2*A*, 3*A*). Beyond five beats, no further increase was observed. The slope value for the curve associated with the response at one beat of stimulation was significantly different from that for five ($P < 0.05$), but not that for two. The respective mean peak bradycardia values are illustrated in Fig. 2*A* and 3*C* for comparison at each increased number of cardiac cycles stimulated. As the number of beats stimulated increased, the peak bradycardia increased until the number stimulated exceeded 40. Thus, the maximum bradycardia elicited from a supramaximal electrical stimulation of the LAN occurred on the average at 47 beats and

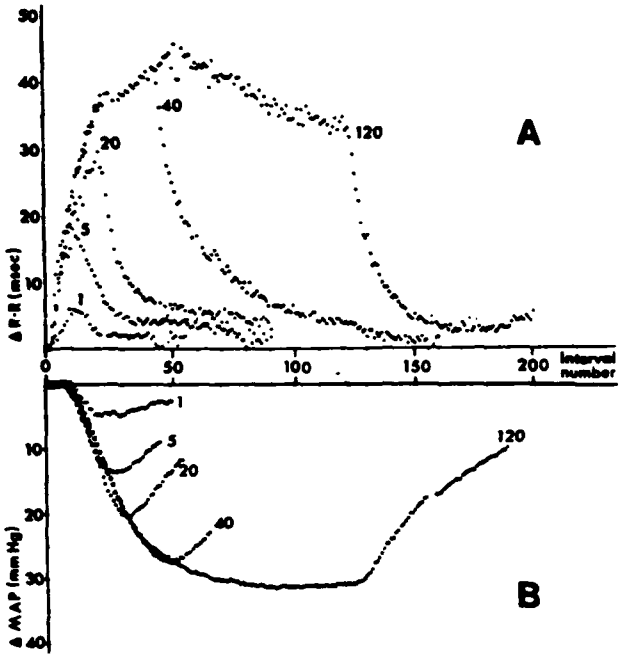

FIG. 2. Oscillograph display of computer-controlled overlays of magnitude and time course of average HR and MAP response when LAN was stimulated for 1, 5, 20, 40, and 120 cardiac cycles in the rabbit (abbreviations same as in Fig. 1). *A:* average bradycardia (ΔR-R) for 1 (n = 5), 5 (n = 8), 20 (n = 6), and 120 (n = 7) beats of LAN stimulation (n = number of animals). *Ordinate:* change in R-R interval time. *Abscissa:* heartbeat number from beginning of stimulus (zero). Stimulation starts at zero for each curve. *B:* average blood pressure fall (Δ MAP) for same animals and number of beats of LAN stimulation as in *A*. *Ordinate:* change in MAP. *Abscissa:* same as Fig. 1. Stimulation starts at zero for each curve.

resulted in a 23% reduction in heart rate from control levels. In Fig. 2*A* it is also apparent that the average bradycardia response curves for 40 and 120 beats of stimulation display an abrupt change in slope or shoulder. The magnitude of the responses at this shoulder was 35.6 ± 8 and 32.8 ± 10.2 ms increase in R-R interval, respectively. These shoulder values are not significantly different from the peak values of their respective curves or the peak value after 20 intervals of stimulation. However, these shoulders were always easy to identify and occurred significantly earlier (17 and 20 intervals) on the respective response curves. The LTP bradycardia values for the various beats of stimulation are listed in Table 1. The LTP values for one, two, and five beats of stimulation were not significantly different from each other. Values for 12, 20, 40, and 120 beats of stimulation were significantly different ($P < 0.05$) from the prior values and from each other (Table 1).

After stimulation, individual time constants were determined for the respective bradycardia recovery curves and not found to be significantly different; the mean of the averages was 2.5 s or 11.5 beats.

The respective curves representing mean blood pressure responses are shown in Fig. 2*B* for the same animals as in Fig. 2*A*. The average of the control MAP values was 84.6 ± 2.8 mmHg (range 81.6–90.9). Onset of hypotension is essentially the same regardless of the

TABLE 1. *Latency to peak bradycardia and decrease in blood pressure from left aortic nerve stimulation*

Stimulus Duration Intervals	n	Latencies			
		Bradycardia, ms	Intervals	Blood pressure, ms	Intervals
1	5	1836 ± 129	9	3733 ± 215	18
2	8	2234 ± 158	10	4924 ± 300	25
5	8	2307 ± 307	10	5106 ± 350	25
12	7	2924 ± 231	12	5445 ± 323	26
20	6	4350 ± 117	20	6619 ± 252	32
40	6	9356 ± 264	41	11234 ± 302	48
120	7	11288 ± 1253	47	18110 ± 1815	91

Values are means ± SE.

number of beats stimulated and occurred between the eighth and ninth beat after stimulus initiation (LTO 1,880 ± 230 ms). Hence, the onset of MAP change is delayed for at least six beats or more than 1,600 ms compared with bradycardia onset. Initial mean slope averages were calculated for each duration of stimulation (Figs. 2*B* and 3*B*). The slopes for one and two beats were significantly less than the initial slope for five stimulated beats or more. No further significant increase in slope was observed when more than five beats were stimulated (Fig. 3*B*). On the average, maximal MAP depression occurred after 90 successive beats of stimulation of the LAN and averaged 28.9 ± 5.1 mmHg or a 35% decrease from control values (Fig. 3*D*). The LTP hypotension averages for the various number of cardiac cycles stimulated are listed in Table 1. The average LTP for 2 beats was significantly different ($P < 0.05$) from 1 but not from five or 12 stimulated beats. The LTP averages for 20, 40, and 120 stimulated beats were progressively and significantly larger ($P < 0.05$) than each prior value respectively. When the LAN had been stimulated for 120 beats, MAP continued to remain maximally depressed for an average of eight beats after the stimulation was stopped. The MAP did not peak until after the end of stimulation when stimulation was confined to 40 beats or less and in all cases recovery was delayed at least 8 beats after stimulation ceased. Blood pressure did not recover as rapidly as heart rate. Time constants were calculated for the recovery part of the blood pressure response curve. No significant difference was found between these time constants and the mean of the averages was 8.5 ± 0.7 s or 45 beats.

Finally, to determine whether or not blood pressure depression during aortic nerve stimulation might be influenced by a fall in cardiac output due to bradycardia, the peripheral end of the sectioned right vagus was stimulated. Electrical stimulation parameters were the same as LAN stimulation except that voltage was adjusted (ranging between 3 and 3.5 V) to obtain a heart rate change similar to that observed during LAN stimulation. As a result of vagal stimulation in two animals, average R-R interval increased 67 ms or 31% over control while MAP fell only 5.7 mmHg or 5.9% below control. Thus, the significant hypotension observed during left aortic nerve stimulation cannot be explained solely as a result of a depressed cardiac output secondary to the bradycardia.

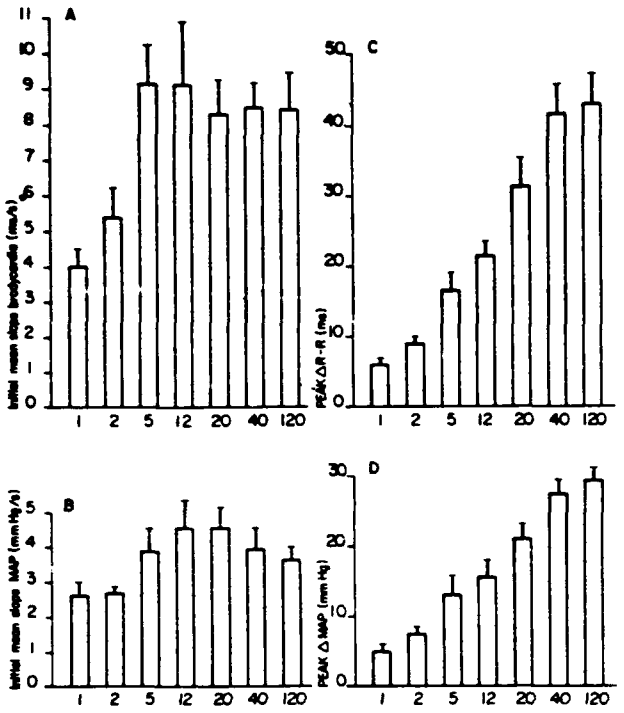

FIG. 3. Mean (bar) ± SE (bracket) of initial mean slope and peak bradycardia and MAP depression values (ordinate) during selected beats of LAN stimulation in rabbit (abscissa). Abbreviations same as in Fig. 1. *A*: bradycardia initial mean slope (ms/s) for 1, 2, 5, 12, 20, 40, and 120 beats of stimulation. All values for 5 beats or more are significantly different from 1 and 2 beats ($P < 0.05$). *B*: mean arterial pressure initial mean slope (mmHg/s) for same animals and beats of stimulation as in *A*. Values for 5 through 40 beats are significantly different from 1 and 2 beats ($P < 0.05$). *C*: peak bradycardia (ms) for same animals and beats of stimulation as in *A*. Maximum reflex bradycardia was observed on average after 47 beats of LAN stimulation. *D*: peak MAP depression (mmHg) for same animals and beats of stimulation as in *A*. Maximum reflex MAP depression was observed on average after 90 beats of LAN stimulation.

DISCUSSION

Supramaximal stimulation of the LAN during one or more cardiac cycles resulted in a reflex reduction in both heart rate and blood pressure. In all animals used, and for any number of beats stimulated, the onset of bradycardia occurred within two beats from the beginning of stimulation and the onset of hypotension six or seven beats later. Therefore, the latency to onset for both responses is dependent on information received from the first beat stimulated irrespective of the number of beats stimulated.

Heart rate response to aortic nerve stimulation in the rabbit that was confined to one cardiac cycle has already been demonstrated (5). Our results have confirmed this finding and have shown that blood pressure too can be influenced in like manner. However, because the blood pressure changes were much later, it is apparent that rapid regulation of systemic pressure is limited by a significant delay in the physiological system.

Previous studies in the rabbit have indicated that the rapid onset of bradycardia involved myelinated afferent fibers and vagal efferent fibers (6, 7). Fiber types involved and possible implications in the slower onset of blood pressure changes have not been investigated. Thus, it is not clear whether the delay in onset of hypotension is the result of a CNS or effector organ process. Interestingly, the onset delay for hypotension observed in this study is similar to the delay in bradycardia onset after vagotomy previously reported (7). In that study, cardiac sympathetic influence appeared to be primarily dependent on alteration of aortic nerve A fiber activity. Douglas et al. (3) suggested that the earliest and most rapid onset of hypotension observed in rabbits resulted from stimulation of small, myelinated aortic nerve A fibers. Thus, myelinated afferents are likely involved in this slow-onset peripheral sympathetic response. In a recent study, de Groat and Lalley (2) indicated that the feline carotid sinus reflex sympathetic conduction times, from afferent through central delay to postganglionic efferent activity, ranged from 72 to 217 ms. On the other hand, Warner and Russell (11) stimulated the canine cardiac sympathetic nerves (primarily postganglionic efferent fibers) and observed 1.5- to 2-s time delays both in the onset of tachycardia and return of the heart rate to control levels. These delays are similar to those associated with blood pressure changes in the present experiments and those of Kardon et al. (7). Hence, the neutral conduction time is relatively rapid and comprises a small portion of the time required for initiation of reflex cardiovascular responses. The major portion of the observed time requirement for reflex blood pressure change appears to involve mechanisms at the effector organ site.

The initial mean slope of both the heart rate and blood pressure responses was not constant when the number of intervals stimulated was altered between one and five. In both responses the slope became progressively steeper as the number of cardiac cycles stimulated was increased up to five. Any number of beats stimulated beyond five did not elicit further change in the slope. Such changes in slope suggest an important reinforcement phenomenon that might function to maximize the rate of response to a persistent change in afferent baroreceptor nerve activity. Whereas brief changes in nerve activity (involving less than five cardiac cycles) would elicit subtle readjustments, changes involving five cycles or more would maximize the rate of response.

In the present study, peak bradycardia in response to stimulation of the LAN for 1, 2, and 5 cardiac cycles occurred at 8, 10, and 10 beats, respectively. Thus, the peak response occurred well after stimulation ceased. However, with stimulation of 12 or more intervals, the peak response occurred at or prior to cessation of stimulation and recovery responses began within 2 beats. At present it is not known what CNS mechanism might be involved to cause continued development of the response after low stimulus durations. Similar nonlinear input-output was noted by Katona and Barnett (8). They observed in the cat and dog a difference between the phasic activity of the baroreceptor fiber and the nonphasic, prolonged activity of the cardiac vagal fiber after imposition of blood pressure changes. It is possible

that baroreceptor afferents initiate a "reverberating activity" in the CNS similar in nature to that hypothesized by Hockman and Talesnik (4), which modifies afferent input resulting in prolonged efferent output after short-duration stimuli. However, to explain the rapid "off" and recovery response observed at longer stimulus durations, an additional mechanism must be considered. One possibility could be that when stimulus duration is extended into the period when blood pressure is depressed, and is detectable by the intact aortic and carotid sinus baroreceptors, attenuation or inhibition of this reverberating modulatory activity results from altered afferent activity from these receptors.

Significant hypotension was not associated with bradycardia produced by efferent vagal stimulation in this study. Hence, the fall in blood pressure during LAN stimulation was primarily due to a reflex decrease in vascular sympathetic tone. If blood pressure was influenced by bradycardia, the relationship was too subtle to be identified by our methods.

Onset and recovery of both heart rate and blood pressure responses were consistent and there was seldom any indication that blood pressure returned toward control during stimulation. This suggests that CNS modulation of sympathetic activity to the peripheral vasculature is sustained as long as the aortic nerve input is sustained in spite of the falling blood pressure. On the other hand, the reflex control of heart rate is more complex, involving simultaneous alterations in both vagal and sympathetic efferent activity.

The onset interval and initial mean slope for reflex bradycardia during the first 10 intervals must be mediated principally through vagal efferents. The LTP for reflex bradycardia after stellectomy in the rabbit has been shown to occur near the 20th interval; after vagotomy with sympathetic nerves intact the LTP was almost doubled (7). That study also demonstrated that the latency to onset of heart rate changes after vagotomy occurred much later, at the eighth or ninth beat, which corresponds to onset of blood pressure responses observed in this study. Thus, in the rabbit, changes in end-organ responses mediated by sympathetic tone seem to have similar temporal characteristics for the heart and vascular areas during aortic afferent stimulation.

Finally, since the abrupt changes in the slope of the heart rate change occurs as blood pressure falls, perhaps the decrease in neural activity from intact baroreceptors influences vagal outflow selectively. This is supported by evidence that the rabbit aortic afferent C fibers do not elicit changes in heart rate through efferent sympathetic pathways, whereas they are important in vagal control of heart rate (7). Furthermore, unmyelinated aortic afferents reportedly are absent from sympathetic sensory areas of the brainstem (9). Thus, changes in afferent C fiber activity from intact baroreceptors may be occurring that influence vagal but not sympathetic outflow.

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REFERENCES

1. CHALMERS, J. P., P. I. KORNER, AND S. W. WHITE. The relative role of the aortic and carotid sinus nerves in the rabbit in the control of respiration and circulation during arterial hypoxia and hypercapnia. *J. Physiol., London* 188: 435–450, 1967.
2. DE GROAT, W. C., AND P. M. LALLEY. Reflex sympathetic firing in response to electrical stimulation of the carotid sinus nerve in the cat. *Brain Res.* 80: 17–40, 1974.
3. DOUGLAS, W. W., J. M. RITCHIE, AND W. SCHAUMANN. Depressor reflexes from medullated and nonmedullated fibres in the rabbits aortic nerve. *J. Physiol., London* 132: 187–198, 1956.
4. HOCKMAN, C. H., AND J. TALESNIK. Central nervous system modulation of baroreceptor input. *Am. J. Physiol.* 221: 515–519, 1971.
5. KARDON, M. B., D. F. PETERSON, AND V. S. BISHOP. Beat-to-beat regulation of heart rate by afferent stimulation of the aortic nerve. *Am. J. Physiol.* 227: 598–600, 1974.
6. KARDON, M. B., D. F. PETERSON, AND V. S. BISHOP. Reflex bradycardia due to aortic nerve stimulation in the rabbit. *Am. J. Physiol.* 225: 7–11, 1973.
7. KARDON, M. B., D. F. PETERSON, AND V. S. BISHOP. Reflex heart rate control via specific aortic nerve afferents in the rabbit. *Circulation Res.* 37: 41–47, 1975.
8. KATONA, P. G., AND G. O. BARNETT. Central origin of asymmetry in the carotid sinus reflex. *Ann. N. Y. Acad. Sci.* 156: 779–786, 1969.
9. KUMADA, M., AND H. NAKAJIMA. Field potentials evoked in rabbit brain stem by stimulation of the aortic nerve. *Am. J. Physiol.* 223: 575–582, 1972.
10. NEIL, E., C. R. M. REDWOOD, AND A. SCHWEITZER. Effects of electrical stimulation of the aortic nerve on blood pressure and respiration in cats and rabbits under chloralose and Nembutal anesthesia. *J. Physiol., London* 109: 392–401, 1949.
11. WARNER, H. R., AND R. O. RUSSELL, JR. Effect of combined sympathetic and vagal stimulation on heart rate in the dog. *Circulation Res.* 24: 567–573, 1969.

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Reduction in Baroreflex Cardiovascular Responses Due to Venous Infusion in the Rabbit

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SUMMARY **We studied reflex bradycardia and depression of mean arterial blood pressure (MAP) during left aortic nerve (LAN) stimulation before and after volume infusion in the anesthetized rabbit. Step increases in mean right atrial pressure (MRAP) to 10 mm Hg did not result in a significant change in heart rate or MAP. After volume loading, responses to LAN stimulation were not as great and the degree of attenuation was proportional to the level of increased MRAP. A change in responsiveness was observed after elevation of MRAP by only 1 mm Hg, corresponding to less than a 10% increase in average calculated blood volume. After an increase in MRAP of 10 mm Hg, peak responses were attenuated by 44% (heart rate) and 52% (MAP), and the initial slopes (rate of change) were reduced by 46% (heart rate) and 66% (MAP). Comparison of the responses after infusion with blood and dextran solutions indicated that hemodilution was an unlikely explanation for the attenuation of the reflex responses. Total arterial baroreceptor denervation (ABD) abolished the volume-related attenuation of the cardiovascular responses, whereas attenuation was still present following bilateral aortic nerve section or vagotomy. It thus appears that the carotid sinus responds to changes in blood volume and influences the reflex cardiovascular responses to afferent stimulation of the LAN. On the other hand, cardiopulmonary receptors subserved by vagal afferents do not appear to be involved.**

VOLUME LOADING, sufficient to raise the venous pressure and dilate the heart, has been observed to produce striking cardiovascular responses.[1-3] Since Bainbridge[3] first reported an increase in heart rate during intravenous infusion many studies have attempted to describe the efferent pathways[4-9] of reflexes originating from receptors located in the cardiopulmonary region.[10-14] Still, the interaction of the low pressure cardiopulmonary receptors with the arterial baroreflex system is not clear.[15] Under conditions of arterial baroreceptor isolation or denervation, the receptors in the cardiopulmonary region that are subserved by afferent vagal fibers have been shown to exert a restraint on the sympathetic adrenergic outflow to the peripheral vasculature in the dog[16] and rabbit.[17] Recently, Vatner et al.[2] found that arterial baroreflex sensitivity in dogs is reduced as atrial pressure is increased by volume loading and suggested that the set point or gain of the arterial baroreflex system is altered during infusion. These alterations might occur at either the receptor site or in the central nervous system. Although recent evidence[18, 19] does suggest that reflex responses from one input are modified by other afferent inputs through integration in the central nervous system, it is unlikely that specific modification of systemic arterial baroreceptors is involved. In the dog, Gupta et al.[20] found dramatic increases in atrial type B receptor activity with volume expansion, while observing only small changes in activity of individual aortic fibers. However, later work by Edis[21] demonstrated that the aortic baroreceptor in the dog shows little tonic activity; consequently, in the dog, one would not expect to see large changes in aortic nerve activity with modest alterations in vascular volume. On the other hand, in the rabbit, Kumada and Sagawa[22] found the aortic baroreceptor nerve activity recorded from multifiber preparations to be proportional to modest blood pressure changes during 20% volume loading and 10% blood loss.

This study was designed to investigate the influence of volume expansion on responses to aortic nerve stimulation in the intact and selectively denervated rabbit. Heart rate and blood pressure responses were measured before and after steady state alterations in mean right atrial pressure (MRAP). The relative influence of low and high pressure receptors was examined and the threshold for atrial pressure necessary to effect altered responses was determined. The rabbit was chosen as the model for study because it has an easily identified aortic nerve which subserves only baroreceptors, and the vascular responses are well defined.[23, 24]

Methods

Twenty-four rabbits weighing between 1.48 and 2.23 kg were anesthetized with sodium pentobarbital (Diabutal, Diamond Laboratories), 30 mg/kg, iv, via an ear vein for

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this study. Supplemental anesthetic was administered through a cannulated femoral vein to maintain a light level of surgical anesthesia. The descending aorta (via the femoral artery) and the right atrium (via the jugular vein) also were cannulated and connected to Statham P23Db and P23Bb strain gauges to record arterial and right atrial pressures, respectively. Heart rate was monitored through sternal needle electrodes connected to a Beckman 9847B cardiotachometer coupler.[24] Blood pressures and heart rate initially were recorded on a Beckman R411 oscillograph with parallel output signals from the arterial pressure and heart rate channels connected to a DEC PDP 8/E digital computer. A tracheostomy was performed and the rabbits were artificially ventilated by the technique published previously[24] to ensure maintenance of normal blood Po_2, Pco_2, and pH. Through a midventral incision, the left and right aortic nerves were located in the cervical region and carefully isolated from surrounding tissue for about 1 cm.[24, 25] In addition, in four rabbits the vagi, and in five rabbits the carotid sinus nerves, also were carefully isolated and looped with a loose thread for identification prior to bilateral sectioning. Loss of the reflex responses due to bilateral carotid occlusion verified carotid sinus denervation.[25] After carotid sinus denervation total arterial baroreceptor denervation (ABD) was considered complete when both aortic nerves also were sectioned. In all rabbits the left aortic nerve (LAN) was sectioned near the sternum and bathed in mineral oil, as previously described[24, 26] The central end of the LAN was placed on bipolar (platinum-iridium) electrodes which were connected to a Grass SD9 stimulator. The stimulator was activated by the Schmitt trigger of the computer which was synchronized with the R wave of the electrocardiogram (ECG). Regulation of the stimulus timing and stimulus parameters, as well as continuous calculation of the length of each R-R interval and beat-to-beat mean arterial pressure (MAP), were accomplished using the computer and special computer program systems. An experimental trial consisted of: (1) 10 successive control cardiac cycles, (2) bursts of electrical stimuli coupled to each R wave of the ECG beginning with the 11th interval and continuing through 120 intervals, and (3) continuous data collection through recovery to control. Each burst of electrical stimulation was made up of 10 rectangular pulses (10 V) delivered 10 msec after the recorded R wave of the ECG. The impulse duration was 0.3 msec, the stimulus frequency was 80 Hz, and burst duration was 113 msec. For each experimental condition data from five or more trials were averaged for each rabbit. The peak change in R-R interval and blood pressure, the latencies to onset and peak responses, as well as the initial slope for each response, were calculated as previously described.[26]

The heart rate and blood pressure responses to LAN stimulation were studied in 14 rabbits infused with dextran in physiologic solution, 5 g/1,000 ml (dextran 40, mol wt 40,000, Pharmacia), heated at 38°C, and one rabbit was infused with whole blood from a heparinized donor. The rate of infusion through the femoral vein was adjusted to ensure a steady rise in right atrial pressure. Infusions were given over a period of 6–14 minutes. Once the MRAP had reached a predetermined level (1, 2.5, 5, or 10 mm Hg) infusion was continued at a rate necessary to maintain MRAP at that level. Trial stimulations of the LAN were initiated after heart rate and MAP reached stabilized steady state values.

Reflex responses to LAN stimulation were also studied in four rabbits which were bled after prior infusion of dextran solution. Each rabbit was bled until the volume of blood removed equaled the volume of dextran solution previously infused. In some instances, slightly more blood was withdrawn to reduce MRAP to control level (range, 0–1.5 mm Hg). In no case did the volume withdrawn plus urine volume collected during this period exceed the previously infused volume by more than 10 ml. Following each bleeding, a period of 1–2 minutes was allowed for stabilization of the rabbit's heart rate and blood pressure before further experimental trials were undertaken.

To monitor the red blood cell concentration, hematocrits were measured before and after each infusion or bleeding. In addition, urine flow and volume also were routinely monitored throughout each experiment.

Values are reported as the mean or mean difference ± the standard error of the mean (SEM). Statistical evaluation was made by use of the appropriate Student's *t*-test for paired or unpaired comparisons. P values (<0.05) were considered significant.

Results

In 14 rabbits MRAP was elevated to 5 mm Hg and in seven of these, to 10 mm Hg, using an average of 41 and 71 ml of dextran solution. This acute volume expansion did not significantly affect either heart rate or blood pressure (Table 1). Representative changes in response to LAN stimulation are shown in Figure 1 before and after step increases in right atrial pressure. The duration of stimulation used (120 cardiac cycles) previously has been shown to produce maximum heart rate and blood pressure responses.[26] Note that following the increases in MRAP, the amplitude of each response was further decreased (Fig. 1). The average maximum increase in R-R interval of 45.4 ± 3.0 msec ($n = 14$) induced by aortic nerve stimulation was significantly reduced ($P < 0.01$) to 31.6 ± 4.2 ($n = 14$) and 25.4 ± 3.2 ($n = 7$) following increases in MRAP of 5 and 10 mm Hg, respectively (Fig. 2A). Similarly, for the same rabbits, the average preinfusion MAP response of −30.0 ± 2.3 mm Hg was reduced to −17.6 ± 2.4 ($P < 0.005$) and −14.3 ± 1.0 ($P < 0.005$), respectively (Fig. 2B). Neither the latency to onset (LTO) nor the latency to peak (LTP) was significantly altered for either reflex response and both were similar to those previously described.[26] However, the initial slopes for both responses (Fig. 2C and D) were diminished with each increase in atrial pressure and this change, in conjunction with the lower maximum responses, would account for the essentially unchanged LTP's. The initial slope of the heart rate response decreased from 9.29 ± 0.88 msec/sec to 5.66 ± 0.84 ($P < 0.01$) and 5.03 ± 0.85 ($P < 0.05$). Similarly, the initial slope of the control MAP response, 3.68 ± 0.34 mm Hg/sec, was decreased to 2.14 ± 0.41 ($P < 0.005$) and 1.25 ± 0.13 ($P < 0.005$) by increases in MRAP of 5 and 10 mm Hg, respectively.

TABLE 1 *Responses to Volume Infusion in Rabbits with and without Vagi Intact*

Group condition	*n*	R-R (msec)	MAP (mm Hg)	Hct (% RBC)	Dextran volume (ml)
Vagi intact					
Control	14	233 ± 10	84.5 ± 6.4	34.9 ± 0.8	
ΔMRAP					
+5 mm Hg	14	236 ± 8	90.0 ± 5.7	22.3 ± 0.9	41.0 ± 2.7
+10 mm Hg	7	233 ± 6	84.5 ± 5.2	16.1 ± 1.2	71.0 ± 8.0
Vagotomized					
Control	4	234 ± 10	83.9 ± 6.3	33.5 ± 0.4	
Vagotomized	4	228 ± 11	86.8 ± 8.6	33.4 ± 0.3	
ΔMRAP					
+5 mm Hg	4	243 ± 14	92.0 ± 7.1	23.9 ± 1.2	42.0 ± 2.9
+10 mm Hg	4	234 ± 11	91.6 ± 6.6	19.5 ± 1.1	63.8 ± 4.7

Values for mean arterial pressure (MAP), R-R interval length, and change in mean right atrial pressure (ΔMRAP) were obtained prior to stimulation. Hematocrits (Hct) represent values immediately after stimulation. All values are mean ± SEM; *n* = number of rabbits.

Reflex responses also were quantitated in seven rabbits prior to and after MRAP increases of 1.0, 2.5, and 5.0 mm Hg, to estimate the threshold of the volume-loading influence. Average control heart rate and MAP, as well as LTO and LTP responses, were not found to be significantly different in these rabbits when compared to those previously studied. Progressive decreases in both average initial slope and peak change were observed for both reflex responses as MRAP was increased (Fig. 2). When MRAP was increased by 1 mm Hg there were significant changes ($P < 0.05$) from control averages in the maximum MAP response (−21.9 ± 2.9 mm Hg) and initial slope (2.82 ± 0.37 mm Hg/sec). The mean heart rate responses also were reduced (peak, 40.5 ± 5.0 msec; initial slope, 8.16 ± 0.94 msec/sec), but these changes were not found to be significant. However, with subsequent increases in MRAP of 2.5 and 5.0 mm Hg, the peak and initial slope for both reflex responses were significantly ($P < 0.05$) reduced. For an increase in MRAP of 2.5 mm Hg the maximum heart rate change was 34.0 ± 3.6 msec and the initial slope was 6.63 ± 0.99 msec/sec; comparable values for the MAP reflex change were: maximum, −18.9 ± 3.3 mm Hg, and initial slope, 2.59 ± 0.36 mm Hg/sec.

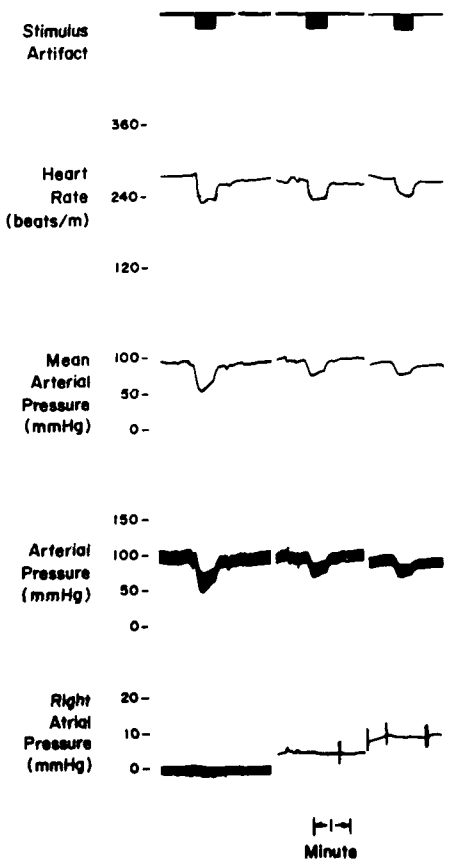

FIGURE 1 *Heart rate, mean arterial and pulse pressure, and right atrial pressure responses to left aortic nerve stimulation in the rabbit, before and during increases of 5 and 10 mm Hg (dextran infusion) in mean right atrial pressure (MRAP), respectively. MRAP and occasional pulse pressure checks were recorded during the infusion.*

Acute volume loading alone resulted in significant hemodilution that was estimated to average a 26% and a 43% increase in vascular fluid volume when MRAP was elevated to 5 and 10 mm Hg, respectively (Table 1). To determine whether hemodilution was responsible for the attenuated responses, bleeding was performed in four rabbits in which the MRAP had first been increased to 10 mm Hg. As shown in Table 2, although after bleeding the hematocrit still was subnormal (20%) the reflex heart rate and MAP responses returned to near control values. To eliminate the dilution factor, whole blood from a donor rabbit was infused into one rabbit. The infusion alone did not alter resting heart rate or MAP. Attenuation of the peak and initial slope of the reflex responses to LAN stimulation (Fig. 3) was similar to that observed with comparable increases in MRAP during dextran infusion (Fig. 2). The similarity in LAN-induced changes in heart rate and MAP during increased MRAP with dextran or blood infusion suggests that these responses are influenced by the degree of volume loading and not the associated hemodilution.

In the dog, acute volume loading with subsequent increases in atrial pressure stimulates receptors with affer-

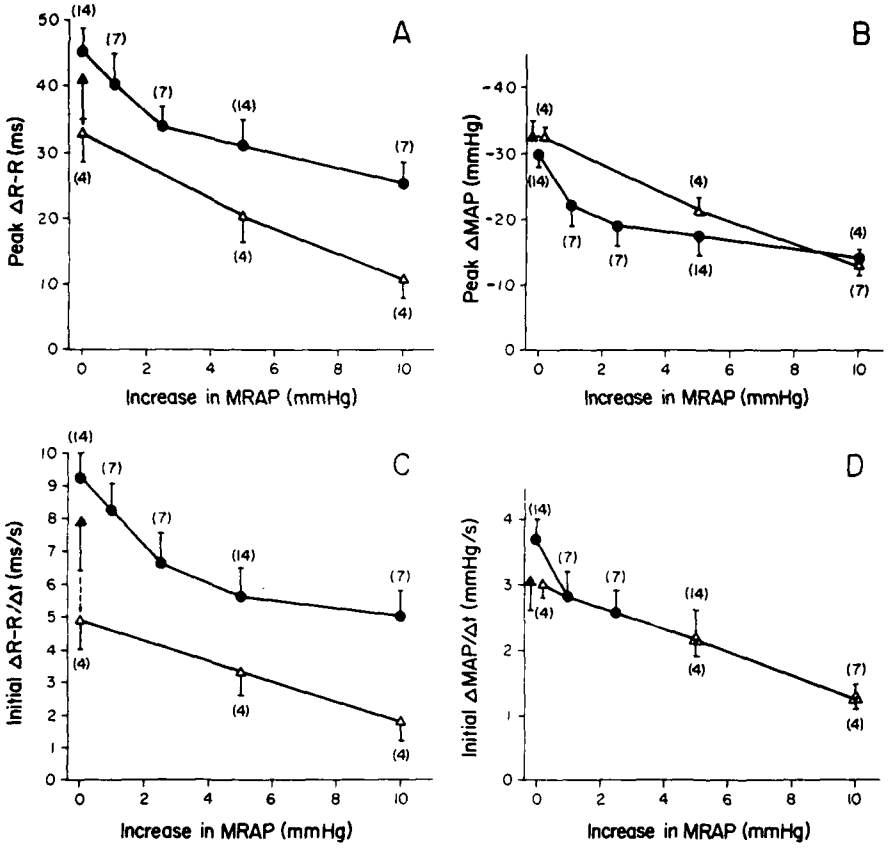

FIGURE 2 *Peak change (Δ) and initial slope (Δ/Δt) of heart rate (R-R interval increase) and mean arterial pressure (MAP) decrease in response to left aortic nerve (LAN) stimulation in the rabbit, prior to and during dextran infusion-induced increases in mean right atrial pressure (MRAP). Solid circles indicate the mean response values for controls; triangles indicate the mean response values pre- (solid) and post- (open) vagotomy. Brackets indicate 1* SEM*: figures in parentheses are the number of rabbits tested at the respective level of increased MRAP. R-R intervals are measured in milliseconds (ms).*

ents in the vagus nerves.[2] Accordingly, the reflex changes in heart rate and MAP were examined after vagotomy in four additional rabbits before and during increases in MRAP. As shown in Table 1, vagotomy alone had little effect on resting heart rate or MAP. In corroboration of previous results,[27] vagotomy significantly ($P < 0.01$) increased the LTO for the reflex bradycardia from an average of 3.3 ± 0.6 (0.75 ± 0.12 sec) beats to 6.3 ± 0.3 (1.44 ± 0.20 sec), but had little effect on the LTO or reflex changes in MAP with LAN stimulation. Vagotomy also reduced the magnitude and slope of the reflex heart rate response (Fig. 2A and C). After vagotomy, increases in MRAP of 5 and 10 mm Hg attenuated the peak reflex increase in R-R interval from 33.5 ± 4.6 msec to 20.4 ± 3.5 ($P < 0.05$) and 10.9 ± 2.6 ($P < 0.025$), respectively. The reflex fall in MAP also was attenuated in the vagotomized rabbits at each level of increased MRAP (Fig. 2B) from a preinfusion average of −32.6 ± 0.9 mm Hg to −21.2 ± 1.7 ($P < 0.025$) and −12.9 ± 1.5 ($P < 0.005$), respectively. The LTO, LTP, and initial slope of the reflex changes in MAP were not significantly different when compared to the respective values from trials with the vagi intact. Thus, while the vagal efferent component of the reflex bradycardia was abolished by vagotomy, the sympathetic component remained intact. After vagotomy the reflex bradycardia mediated by the intact sympathetics, as well as the reflex fall in MAP, were attenuated in a

TABLE 2 *Assessment of Hemodilution on the Cardiovascular Reflex Responses to Aortic Nerve Stimulation: Volume Infusion followed by Bleeding in Four Rabbits*

Property	Control (MRAP ≤ 0–1 mm Hg)	Infusion (MRAP ≥ +10 mm Hg)	Bleeding (MRAP ≤ 0.0 mm Hg)
Hct (%)	34.8 ± 1.0	13.9 ± 1.1*	20.0 ± 1.3*
Initial R-R (msec)	239 ± 18	234 ± 14	226 ± 10
Initial MAP (mm Hg)	84.1 ± 5.4	86.8 ± 3.3	78.8 ± 3.2*
LAN stimulation			
Peak ΔR-R (msec)	40 ± 7	33 ± 5*	39 ± 5
Peak ΔMAP (mm Hg)	28 ± 4	13 ± 2*	28 ± 5

MRAP = mean right atrial pressure; LAN = left aortic nerve; MAP = mean arterial pressure; values are mean ± 1 SEM.
* $P < 0.05$ (paired analysis) for values significantly different from control.

manner similar to that seen prior to vagotomy. Therefore, since the magnitudes and initial slopes of the reflex responses were affected similarly by volume loading in both the intact and vagotomized rabbits, cardiopulmonary receptors subserved by vagal afferents do not seem to be involved in the observed alterations of LAN-invoked reflex changes during intravenous infusion.

To determine the possible contribution of intact arterial baroreceptors to the volume-induced attenuation of the reflex responses, bilateral aortic arch and carotid sinus denervations were performed in rabbits with intact vagi. Prior to total denervation, both aortic nerves were sectioned in two rabbits. Reflex responses to LAN stimulation were found not to be altered either before or after volume loading (MRAP = 5.0 mm Hg) when compared to control responses. In five rabbits, responses to LAN stimulation were quantitated prior to and after total arterial baroreceptor denervation (ABD) and subsequently after volume loading (MRAP = 5.0 mm Hg) of the denervated rabbits. Average control heart rate was not significantly altered by ABD, even though average resting MAP values were increased by 30 mm Hg following ABD (Table 3). The LTO's and initial slopes were not significantly changed following ABD, but the magnitude of the peak and the LTP for the MAP response were both increased significantly (Table 3). After ABD, attenuation of the reflex responses to LAN stimulation by volume loading was not observed. Three rabbits were bled after ABD and volume loading in order to return MRAP and MAP to preinfusion levels. Subsequently, the vagi were sectioned in these rabbits. Although MAP increased on the average by 14 mm Hg following vagotomy and by an additional 19 mm Hg following infusion (MRAP = 5.0 mm Hg), no significant change in heart rate or MAP responses to LAN stimulation were observed when these responses were compared to those obtained prior to vagotomy.

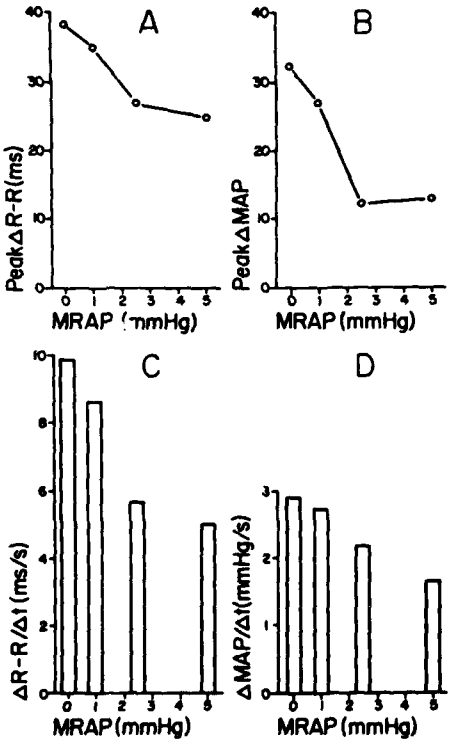

FIGURE 3 *Peak change (Δ) and initial slope (Δ/Δt) of heart rate (R-R interval increase) and mean arterial pressure (MAP) decrease in response to left aortic nerve (LAN) stimulation in one rabbit, prior to and with increases in mean right atrial pressure (MRAP) caused by infusion of whole blood. R-R intervals are measured in milliseconds (ms).*

TABLE 3 *Heart Rate and Mean Arterial Blood Pressure (MAP) Changes Due to Left Aortic Nerve (LAN) Stimulation before and after Arterial Baroreceptor Denervation and following Venous Infusion in Five Rabbits*

Condition	Control (MRAP 0.5 mm Hg)	Arterial baroreceptor denervated: MRAP 0.5 mm Hg	Arterial baroreceptor denervated: MRAP 5.0 mm Hg
Prestimulation			
R-R interval (msec)	221±11	221±6	218±3
MAP (mm Hg)	94±3	124±10*	123±7*
Stimulation-reflex changes			
Heart			
Peak interval Δ (msec)	59±13	71±19	79±15
ΔR-R/Δt (msec/sec)	13.8±3.4	13.0±4.6	12.7±4.4
LTP (interval no.)	43±8	71±23	66±21
MAP			
Peak Δ (mm Hg)	37±4	57±6*	59±6*
ΔMAP/Δt (mm Hg/sec)	5.4±0.5	6.7±0.8	7.1±0.6
ΔLTP (interval no.)	45±4	73±13*	71±5*

MRAP = mean right atrial pressure; LTP = latency to peak; values are mean or mean difference ± 1 SEM.
* Significant ($P < 0.05$) change from control (paired analysis).

Discussion

This study has demonstrated that acute volume loading depresses the reflex cardiovascular responses to electrical activation of the rabbit aortic nerve. The mechanism responsible for this attenuation was highly sensitive, since identifiable influences occurred after an elevation in MRAP of only 1.0 mm Hg, or a vascular volume expansion of less than 10%. Although there was significant hemodilution when MRAP was elevated by acute volume loading (Table 1), hemodilution could not explain the attenuated cardiovascular responses to LAN stimulation. Hemorrhage after volume loading caused total fluid volume, as well as the responses to nerve stimulation, to equal preinfusion values even though significant hemodilution remained (Table 2). Furthermore, an attenuation of the reflex changes during LAN stimulation was observed during infusion of blood which did not change the hematocrit (Fig. 3).

Infusion alone did not elicit any significant change in heart rate or MAP even when MRAP was increased by 10 mm Hg. This finding is contrary to results obtained for the conscious dog which indicated that volume loading, sufficient to raise mean atrial pressure to 10 mm Hg or more, produced an increase in both heart rate and MAP.[1, 2] Horwitz and Bishop[1] concluded that in the conscious dog

tachycardia during volume loading was due primarily to reflex inhibition of vagal efferent activity. The control heart rates for rabbits in our present study ranged from 223 to 300 beats/min; these values are similar to those found for the conscious rabbit[18] but considerably higher than the resting heart rate of the conscious dog.[1, 2] Since vagotomy did not (Table 1) result in a sustained change in heart rate above control levels it is likely that the tonic cardiac vagal restraint is slight in the rabbit. Consequently, changes in heart rate during volume loading would be minimal.

There is good evidence for receptors subserved by vagal afferents that are responsive to volume or tension changes in the cardiopulmonary region and that reflexly alter cardiac sympathetic activity.[7, 12, 13] Additionally, in the dog[16] and in the rabbit[17] after sinoaortic denervation, vagal block or section results in a rise in arterial pressure. Clement et al.[28] found in the anesthetized denervated rabbit a 41% decrease in renal sympathetic activity associated with a 10% increase in blood volume. In the dog, vagally mediated cardiopulmonary receptors have been shown to oppose the vasoconstriction due to carotid sinus hypotension more effectively in the kidney than in the hindlimb.[29] Some interaction apparently occurs in the central nervous system between the arterial baroreceptors and the cardiopulmonary receptors, since the magnitude of the canine pressor response to vagal cold block is altered by input from the carotid sinus.[16] A recent study[2] using conscious dogs has suggested that the normal arterial baroreceptor restraint on heart rate is diminished during volume loading, since in spite of a substantial increase in arterial pressure a significant tachycardia occurred and the heart rate responses were unaltered by ABD. Although this study also found the reflex baroreceptor-heart rate response to a pressor agent to be diminished, it did not evaluate the sensitivity of these receptors in control of systemic pressure. These investigators[2] postulated that inputs from the low pressure cardiopulmonary receptors may modify the arterial baroreceptor control of the cardiovascular system. Our results for the anesthetized rabbit do not support this postulate. The inhibitory influence of volume loading on both reflex MAP and heart rate responses to LAN stimulation were unaltered by vagotomy (Fig. 2). Thus, during volume loading, cardiopulmonary receptors subserved by vagal afferents did not play a role in modifying the reflex responses to LAN stimulation. In addition, although volume loading reduced the efferent sympathetic inhibition due to LAN stimulation, the parasympathetic component of the reflex heart rate response was apparently unaltered. This last conclusion is based on the observation that the peak change in the initial slope of the heart rate responses were uniformly reduced after vagotomy when comparisons were made at each level of MRAP tested (Fig. 2A and C).

Following total ABD or ABD plus vagotomy, the reflex cardiovascular responses to LAN stimulation were no longer attenuated by volume expansion (Table 3). Earlier, Kumada and Sagawa[22] suggested that variations in blood volume in the rabbit can be detected by aortic baroreceptors via the small associated changes in arterial pressure. Since the vagally mediated cardiopulmonary receptors were not found to play an important role in the attenuation of the reflex responses caused by infusion, two possible mechanisms were considered. The first involved activation of sympathetic afferents subserving cardiopulmonary receptors[8, 9, 14] and the second activation of arterial baroreceptor afferents from carotid sinus and aortic arch regions responsive to volume loading. By progressive bilateral denervation it was considered that the relative contribution of each mechanism could be distinguished. When both aortic nerves were sectioned prior to carotid sinus baroreceptor denervation, no significant change in the attenuation of the reflex responses was detected. Subsequent to total ABD or ABD plus vagotomy the attenuation observed after infusion was eliminated. Were sympathetic afferents from cardiopulmonary receptors involved, some measurable attenuation would have been observed following ABD. A rise in arterial blood pressure was seen following vagotomy and volume loading in the rabbits after ABD; this indicates that vagally mediated receptors can modify blood pressure in the denervated animal, as reported by others.[15–17, 28, 29]

These results suggest that the carotid sinus baroreceptors detect subtle changes in arterial pressure or volume during infusion and this results in a diminished sympathetic efferent activity to the effector organ. Thus, as previously demonstrated,[30] use of the absence of a change in heart rate or arterial blood pressure as a criterion for the absence of a change in carotid baroreceptor activity during an experimental intervention may lead to inaccurate conclusions.

References

1. Horwitz LD, Bishop VS: Effect of acute volume loading on heart rate in the conscious dog. Circ Res **30:** 316–321, 1972
2. Vatner SF, Boettcher DH, Heyndrickx GR, McRitchie RJ: Reduced baroreflex sensitivity with volume loading in conscious dogs. Circ Res **37:** 236–242, 1975
3. Bainbridge FA: Influences of venous filling upon the rate of the heart. J Physiol (Lond) **50:** 65–84, 1915
4. Brown AM, Malliani A: Spinal sympathetic reflexes initiated by coronary receptors. J Physiol (Lond) **212:** 685–705, 1971
5. Gupta PD: Spinal autonomic afferents in elicitation of tachycardia in volume infusion in the dog. Am J Physiol **229:** 303–308, 1975
6. Karim F, Kidd C, Malpus CM, Penner PE: The effects of stimulation of the left atrial receptors on sympathetic efferent nerve activity. J Physiol (Lond) **227:** 243–260, 1972
7. Koizumi K, Ishikawa T, Nishino H, Brooks CMcC: Cardiac and autonomic system reactions to stretch of the atria. Brain Res **87:** 247–261, 1975
8. Bishop VS, Lombardi F, Malliani A, Pagani M, Recordati G: Reflex sympathetic tachycardia during intravenous infusions in chronic spinal cats. Am J Physiol **230:** 25–29, 1976
9. Uchida Y: Afferent sympathetic nerve fibers with mechanoreceptors in the right heart. Am J Physiol **228:** 223–230, 1975
10. Chevalier PA, Weber KC, Lyons GW, Nicoloff DM, Fox IJ: Hemodynamic changes from stimulation of left ventricular baroreceptors. Am J Physiol **227:** 719–728, 1974
11. Coleridge JCG, Linden RJ: Effect of intravenous infusions upon the heart rate of the anaesthetized dog. J Physiol (Lond) **128:** 310–319, 1955
12. Furnival CM, Linden RJ, Snow HM: Reflex effects on the heart of stimulating left atrial receptors. J Physiol (Lond) **218:** 447–463, 1971
13. Ledsome JR, Linden RJ: A reflex increase in heart rate from distension of the pulmonary-vein-atrial junctions. J Physiol (Lond) **170:** 456–473, 1964
14. Malliani A, Recordati G, Schwartz PJ: Nervous activity of afferent cardiac sympathetic fibers with atrial and ventricular endings. J Physiol (Lond) **229:** 457–469, 1973
15. Pelletier CL, Shepherd JT: Circulatory reflexes from mechanoreceptors in the cardio-aortic area. Circ Res **33:** 131–138, 1973
16. Mancia G, Donald DE, Shepherd JT: Inhibiton of adrenergic outflow to peripheral blood vessels by vagal afferents from the cardiopulmo-

nary region in the dog. Circ Res **33:** 713–721, 1973
17. Pillsbury HRC III, Guazzi M, Preis ED: Vagal afferent depressor nerves in the rabbit. Am J Physiol **217:** 768–770, 1969
18. Korner PI, Shaw J, West MJ, Oliver JR, Hilder RG: Integrative reflex control of heart rate in the rabbit during hypoxia and hyperventilation. Circ Res **33:** 63–73, 1973
19. Kumada M, Nogami K, Sagawa K: Modulation of carotid sinus baroreceptor reflex by sciatic nerve stimulation. Am J Physiol **228:** 1535–1541, 1975
20. Gupta PD, Henry JP, Sinclair R, Von Baumgarten R: Responses to atrial and aortic baroreceptors to nonhypotensive hemorrhage and to transfusion. Am J Physiol **211:** 1429–1437, 1966
21. Edis AJ: Aortic baroreflex function in the dog. Am J Physiol **221:** 1352, 1971
22. Kumada M, Sagawa K: Aortic nerve activity during blood volume changes. Am J Physiol **218:** 961–965, 1970
23. Neil E, Redwood CRM, Schweitzer A: Effects of electrical stimulation of the aortic nerve on blood pressure and respiration in cats and rabbits under chloralose and Nembutal anesthesia. J Physiol (Lond) **109:** 392–401, 1949
24. Kardon MB, Peterson DF, Bishop VS: Reflex bradycardia due to aortic nerve stimulation in the rabbit. Am J Physiol **225:** 7–11, 1973
25. Chalmers JP, Korner PI, White SW: The relative roles of the aortic and carotid sinus nerves in the rabbit in the control of respiration and circulation during arterial hypoxia and hypercapnia. J Physiol (Lond) **188:** 435–450, 1967
26. Stinnett HO, Peterson DF, Bishop VS: Heart rate and blood pressure responses to synchronized stimulation of the aortic nerve. Am J Physiol **230:** 1374–1378, 1976
27. Kardon MB, Peterson DF, Bishop VS: Reflex heart rate control via specific aortic nerve afferents in the rabbit. Circ Res **37:** 41–47, 1975
28. Clement DL, Pelletier CL, Shepherd JT: Role of vagal afferents in the control of renal sympathetic nerve activity in the rabbit. Circ Res **31:** 824–830, 1972
29. Mancia G, Shepherd JT, Donald DE: Role of cardiac pulmonary and carotid mechanoreceptors in the control of hindlimb and renal circulation in dogs. Circ Res **37:** 200–208, 1975
30. Peterson DF, Kaspar RL, Bishop VS: Reflex tachycardia due to temporary coronary occlusion in the conscious dog. Circ Res **32:** 652–659, 1973

Effect of regional myocardial ischemia on cardiac pump performance during exercise

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HORWITZ, LAWRENCE D., D. FRED PETERSON, AND VERNON S. BISHOP. *Effect of regional myocardial ischemia on cardiac pump performance during exercise*. Am. J. Physiol. 234(2): H157-H162, 1978 or Am. J. Physiol.: Heart Circ. Physiol. 3(2): H157-H162, 1978. – The effect of brief periods of regional ischemia upon left ventricular pump performance was studied in nine dogs standing quietly at rest and during running exercise on a treadmill. Transient occlusions of the left circumflex coronary artery resulted in increase in heart rate at rest (+30 beats/min) but not during exercise. Other changes due to occlusion were similar at rest and during exercise and included decreases in stroke volume (−25% standing, −23% running); in dP/dt max, the maximum first derivative of the left ventricular pressure (−20% standing or running); and in left ventricular peak systolic pressure (−13% standing, −21% running); and rises in left ventricular end-diastolic pressure (+4.5 mmHg standing, +6.3 mmHg running). Cardiac output was unchanged by occlusions at rest but fell (−18%) during occlusions while the dogs were running. Propranolol reduced absolute levels of cardiac performance during exercise occlusions but had no effect at rest. Inotropic agents with ischemia had some effects at rest but did not alter exercise hemodynamics. It is concluded that integrated left ventricular function during ischemia is not impaired by exercise, probably because of beta-adrenergic stimulation of nonischemic myocardium.

coronary occlusion; cardiac output; propranolol; ouabain; mannitol

REGIONAL MYOCARDIAL ISCHEMIA reduces the ability of the left ventricle to pump blood (2, 4, 6). Because contractile function of the ischemic portion is impaired, the rate of pressure development and the ejection fraction of the ventricle are reduced (2, 10). Consequently, stroke volume falls and maintenance of cardiac output at normal levels is dependent upon an increase in heart rate (13).

It is a reasonable assumption that local myocardial oxygen demand is a major factor influencing the extent to which regional ischemia disturbs cardiac pump performance. Accordingly, the substantial increase in myocardial oxygen consumption with strenuous physical activity might be expected to exacerbate the deleterious effects of ischemia. Of interest in this regard is a report that in global ischemia of the entire left ventricle due to stenosis of the left main coronary artery, exercise caused severe deterioration in the rate of left ventricular pressure development and the rate and extent of shortening of left ventricular diameter (14). However, when only a portion of the ventricle is ischemic, the responses could differ both qualitatively and quantitatively because of the presence of some normally perfused left ventricular myocardium and, possibly, because of more effective collateral flow channels to the region in which the normal vascular supply is damaged (11). A related question is whether a drug that reduces myocardial oxygen consumption, such as a beta-adrenergic blocking agent, might be expected to exert a salutary effect upon cardiac pump function during regional ischemia either with rest or exercise.

However, in the face of myocardial regional perfusion abnormalities, beta-adrenergic blocking agents could be harmful since the normal sympathetic-mediated augmentation in contractile force generation and pump performance which accompanies exercise (7, 8) would be blocked. On the other hand, it is not known whether an independent increase in contractile function due to pharmacological agents would be beneficial in counteracting ischemic impairment of stroke volume either at rest or during exercise.

The purpose of this study was to evaluate the disturbance in integrated pump performance of the left ventricle due to transient occlusion of the left circumflex coronary artery in conscious dogs at rest and while running on a treadmill. In addition, the extent to which responses to ischemia were modified by beta-adrenergic blockade with propranolol and two agents that augment myocardial contractile force – ouabain and hypertonic mannitol (1) – was assessed under both resting and exercise conditions.

METHODS

Nine mongrel dogs, weighing 14.5–27.3 kg, were trained to run on a level treadmill. After the training period, each dog underwent a sterile thoracotomy under sodium pentobarbital anesthesia. A solid-state pressure transducer (Konigsberg P18) was implanted within the left ventricle, an electromagnetic flow probe was placed around the proximal portion of the ascending aorta, and 18-gauge polyvinyl catheters were inserted into the left atrial appendage, the left jugular vein, and via the left internal mammary artery, into the aorta (7). The left circumflex coronary artery was isolated near its origin, and a polyvinyl occlusive device (2) was placed

around the vessel. Care was taken to avoid damage to the nerve supply of the artery. The electrical leads, catheters, and distal end of the occlusive device were exteriorized at the back of the neck. Three weeks were allowed for recovery from surgery and retraining. At the time of study, each dog could exercise at levels attained prior to surgery.

Aortic flow was measured with a Zepeda square-wave electromagnetic flowmeter. The flow probes were calibrated in vitro before implantation and rechecked after the animals were sacrificed; the two calibrations always agreed within 5%. Flow in late diastole was assumed to be zero. Stroke volume was obtained by integration of the flow signal, with an active circuit, during each ejection period. The frequency response of the flowmeter was limited only by the response of the recorder which was flat to 80 Hz.

The solid-state pressure transducers were precalibrated. Sensitivity was stable during the period of implantation. Small amounts of day-to-day zero drift were corrected by setting the left ventricular end-diastolic pressure equal to the mean left atrial pressure at the beginning of each study while the dogs were at rest (9). The natural frequency of the solid-state transducers has been reported to be in excess of 3,000 Hz (9). The first differential of the left ventricular pressure (dP/dt) was obtained with an active circuit which was linear to 70 Hz. Mean aortic pressure was measured via the implanted catheter with a Statham P23 Db manometer. All signals were inscribed on a Beckman RM oscillograph and an Ampex FR 1300 magnetic tape recorder.

The occlusive device on the left circumflex coronary artery was inflated by injection of saline. Collapse of the vessel and discoloration of tissue distal to the occlusion were observed when the device was tested at surgery. After experiments were completed, all hearts were carefully inspected when the animals were sacrificed, and no evidence of tissue damage was noted in the region distal to the occluder.

Resting measurements were obtained with the dog standing quietly on the treadmill. After control data were measured, the left circumflex coronary artery was occluded for 50 s and then released. Five minutes later, by which time hemodynamics had returned to the initial resting levels, the treadmill was started with the dog running at a speed between 6 and 8 mph. The speed was preselected as one which the particular dog could accomplish consistently, yet appeared to require strenuous effort. Three minutes after running began, the left circumflex coronary artery was again occluded for 50 s. In each case the dog continued to run at the same speed during occlusion. The occluder was then released, and the treadmill was gradually slowed and then stopped. After one or two "control" studies had been obtained without drug intervention in all nine dogs, on later experimental days seven dogs were studied 15 min after administration of propranolol (1.0 mg/kg iv). The exercise level and duration were the same as in the control study. On separate days, five dogs received ouabain (0.025–0.030 mg/kg iv) and were restudied 15 min later. As a third intervention, 3–4 days later, five dogs received mannitol, as a 25% solution, at a dosage of 1.25 mg/kg iv, administered over approximately 12 min and were studied immediately thereafter. Arterial blood samples were drawn for determination of serum osmolality prior to the rest occlusion. Osmolalities were measured by freezing-point determination with an Advanced Instruments osmometer.

Preliminary data established that a steady state was present between 2 and 4 min of running 6–8 mph without occlusion and between 40 and 70 s after occlusion at rest or during exercise; during these periods, heart rate, stroke volume, peak systolic pressure, and dP/dt max varied by less than 5% at end-expiration and showed no discernible trend toward change. The dosage of propranolol used abolished the hemodynamic effects of isoproterenol, 5 μg/min iv, between 15 and 45 min after the propranolol was given. Data were obtained for analysis immediately prior to the onset of occlusions and during the last few seconds of each occlusion. The results of eight consecutive beats were averaged to reduce the effects of respiratory variation or atypical beats (7, 8). Statistical analyses were performed by the paired t-test, using each animal as its own control, and by an analysis of variance for comparison of results with drugs versus control studies.

RESULTS

Effect of Occlusion at Rest and During Exercise

The results of acute occlusion of the left circumflex coronary artery in nine dogs are shown in Table 1. A typical record is shown in Fig. 1. At rest, occlusion resulted in statistically significant increases in heart rate (+30 beats/min) and left ventricular end-diastolic pressure (+4.5 mmHg). Decreases occurred in stroke volume (−8.2 ml), peak systolic left ventricular pressure (−18 mmHg), and the maximum first derivative of the left ventricular pressure, dP/dt max, (−664 mmHg/s). Mean aortic pressure fell in four of the six dogs in which it was measured (−11 mmHg), but this change was not statistically significant. Cardiac output and systemic vascular resistance did not change significantly, nor were there any consistent trends in these parameters.

Without occlusion, as described previously (7), exercise was associated with substantial increases in heart rate, left ventricular systolic pressure, dP/dt max, and left ventricular end-diastolic pressure (Table 1 and Fig. 1). Coronary occlusion during exercise caused a significant rise in left ventricular end-diastolic pressure (+6.3 mmHg) and decreases in stroke volume (−7.7 ml), peak systolic left ventricular pressure (−29 mmHg), and dP/dt max (−920 mmHg/s). In contrast with resting occlusion results, there was no significant change in heart rate but a significant fall in cardiac output (−1,220 ml/min). Mean aortic pressure fell (−18 mmHg), but systemic vascular resistance was not altered. Thus, the major difference between the effects of occlusion at rest and during exercise was that in the presence of similar falls in stroke volume, cardiac output was maintained at rest through an increase in heart rate; but during

TABLE 1. *Effect of coronary occlusion at rest and during exercise in nine dogs*

	Heart Rate, beats/min		Stroke Volume, ml/beat		Cardiac Output, liters/min		dP/dt max, mmHg/s		LVSP, mmHg		LVEDP, mmHg		MAP, mmHg	
	CON	OCC	CON	OCC	CON	OCC	CON	OCC	CON	OCC	CON	OCC	CON	OCC
							Rest							
$\bar{x}$	85±5	125±10	34.3±2.1	26.2±2.1	3.20±0.20	3.21±0.28	3,171±149	2,507±142	130±7	113±7	3.1±1.0	7.5±1.1	96±7	85±7
$\bar{d}$	39.6±7.2		8.2±1.1		0.09±0.09		664±92		17.7±3.7		4.5±1.1		10.6±5.9	
P	<0.01		<0.001		NS		<0.001		<0.01		<0.01		NS	
							Exercise							
$\bar{x}$	186±9	210±16	34.8±2.3	27.1±2.1	6.71±0.40	5.49±0.36	4,815±345	3,894±317	166±10	136±10	7.6±2.0	13.9±6.3	120±11	102±13
$\bar{d}$	12.0±9.0		7.7±1.2		1.22±0.23		920±234		29.9±8.3		6.3±1.3		18.2±8.2	
P	NS		<0.001		<0.01		<0.01		<0.01		<0.01		NS	

CON, preocclusion value; OCC, value at 60 s of occlusion; dP/dt max, maximum first derivative of the left ventricular pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure. $\bar{x}$, mean ±SE for each group. $\bar{d}$, mean difference ±SE occlusion vs. preocclusion. NS, $P \geq 0.05$. All values represent data from 9 dogs except MAP values, which represents 6 dogs.

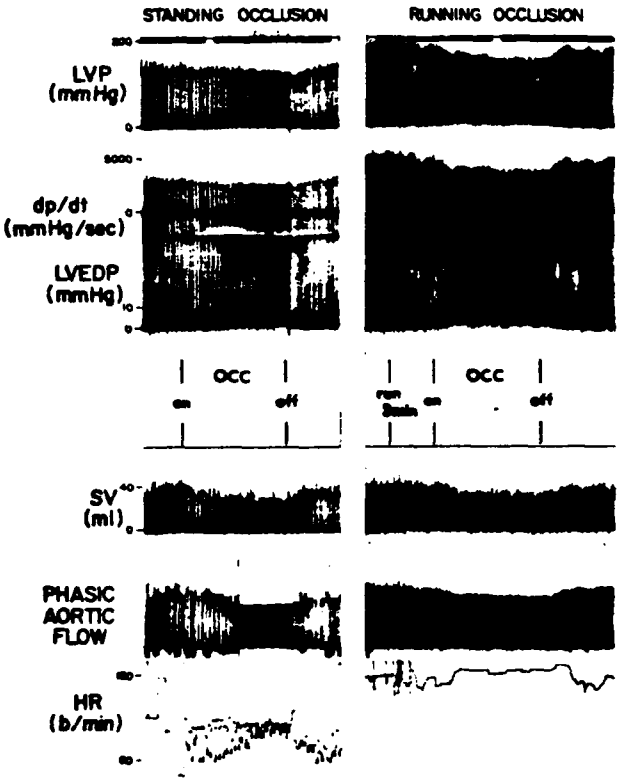

FIG. 1. Portions of a slow-speed recording in an experiment without administration of drugs. High-speed records were used to read left ventricular end-diastolic pressure from magnified left ventricular pressure (LVEDP) and to check other parameters. SV, stroke volume or integral of phasic aortic flow curve, which is shown without units; HR, heart rate; LVP, unmagnified left ventricular pressure; dP/dt, first derivative of left ventricular pressure.

exercise, cardiac output fell because heart rate was unchanged in most animals.

Effect of Drugs

Beta-adrenergic blockade. Propranolol, given to seven dogs, decreased dP/dt max by 594 mmHg and increased left ventricular end-diastolic pressure by 3.6 mmHg at rest. During occlusions at rest, there were no significant differences from results without drugs in the same dogs (Table 2).

Propranolol altered the responses to exercise. The average increment in heart rate was reduced by 33 beats/min. Likewise, the increase in cardiac output was 1.68 liters/min less and in dP/dt max was 772 mmHg/s less than control. Left ventricular end-diastolic pressure rose +3.2 mmHg more with propranolol (Table 2).

The magnitude of the changes due to occlusion during exercise after propranolol were similar to those that occurred at rest after propranolol. However, the absolute levels of heart rate, cardiac output, dP/dt max, and systolic pressure were significantly reduced during exercise with propranolol, whereas end-diastolic pressure was elevated (Table 2).

Ouabain. Results with ouabain in five dogs are shown in Table 3. At rest prior to occlusion, ouabain increased dP/dt max by 511 mmHg/s and left ventricular systolic pressure by 10.8 mmHg. During occlusions at rest, the fall in dP/dt max was less by 451 mmHg/s and systolic pressure was less by 9.4 mmHg, when compared to results without drugs in the same dogs. During exercise, ouabain slowed heart rate by 32 beats/min prior to occlusion but had no other effects either before or during ischemia. On analysis of the magnitudes of the changes due to occlusion, no significant differences were noted between results with ouabain pretreatment and results without drugs.

Mannitol. Osmolality rose by 17 ± 6 (SE) mosmol in response to the infusions of mannitol. At rest, prior to occlusions, elevations in stroke volume (+6.2 ml), cardiac output (+0.73 liter/min), left ventricular end-diastolic pressure (+4.8 mmHg), and left ventricular systolic pressure (+11.8 mmHg) occurred (Table 4). Maximum dP/dt rose in four of six dogs, but this change was not statistically significant. During occlusions at rest, the only significant change was a higher left ventricular end-diastolic pressure (+10.4 mmHg) than that which occurred during occlusions without drugs in the same five dogs (Table 4). During exercise, there were no

TABLE 2. *Effect of propranolol on response to coronary occlusions*

	Heart Rate, beats/min			Stroke Volume, ml/beat			Cardiac Output, liters/min			dP/dt max, mmHg/s			LVSP, mmHg			LVEDP mmHg		
	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ
Rest																		
No drugs	96	125	+29	35.4	26.6	−8.8	3.39	3.25	−0.14	3,171	2,543	627	123	107	−16	3.6	7.8	+4.1
Propranolol	67	111	+25	34.6	26.4	−8.2	2.02	2.87	−0.05	2,577	2,156	−416	116	104	−14	6.9	10.5	+3.6
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	NS	<0.01	NS	NS	NS	<0.05	NS	NS
Exercise																		
No drugs	196	205	+10	26.4	27.9	−6.5	7.03	5.70	−1.33	4,751	3,794	−957	161	128	−33	6.8	12.5	+5.6
Propranolol	163	171	+8	32.8	24.8	−8.0	5.35	4.25	−1.10	2,979	2,295	−684	126	105	−33	11.7	17.1	+5.4
P	<0.01	<0.01	NS	NS	NS	NS	<0.01	<0.01	NS	<0.01	<0.01	NS	<0.05	<0.01	NS	<0.05	<0.05	NS

PRE, mean value immediately prior to occlusion; OCC, mean value during occlusion; Δ, difference between PRE and OCC values. *P* values are for paired *t*-test comparing values with and without drug. NS, $P \geq 0.05$.

TABLE 3. *Effect of ouabain on response to coronary occlusions*

	Heart Rate, beats/min			Stroke Volume, ml/beat			Cardiac Output, liters/min			dP/dt max, mmHg/s			LVSP, mmHg			LVEDP, mgHg		
	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ
Rest																		
No drugs	93	114	+22	36.4	26.6	−9.8	3.14	3.00	−0.14	3,072	2,358	−715	125	113	−11	4.3	8.3	+4.0
Ouabain	88	108	+19	33.5	26.6	−6.2	2.95	2.87	−0.08	3,584	2,808	−775	135	122	−12	6.7	11.1	+4.4
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05	<0.05	NS	<0.05	<0.05	NS	NS	NS	NS
Exercise																		
No drugs	192	195	+3	36.0	28.8	−7.2	6.79	5.61	−1.18	4,761	4,090	−671	163	137	−26	6.7	11.1	+4.4
Ouabain	161	178	+17	37.0	29.1	−7.9	5.95	5.18	−0.77	4,623	3,892	−731	166	148	−17	5.8	14.7	+8.9
P	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

For symbols and abbreviations see footnote to Table 2.

TABLE 4. *Effect of mannitol on response to coronary occlusion*

	Heart Rate, beats/min			Stroke Volume, ml/beat			Cardiac Output, liters/min			dP/dt max, mmHg/s			LVSP, mmHg			LVEDP, mmHg		
	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ	PRE	OCC	Δ
Rest																		
No drugs	90	116	+26	32.2	24.2	−8.0	2.91	2.74	−0.17	3,193	2,668	−525	140	123	−17	4.2	6.7	+2.5
Mannitol	96	122	+26	38.4	27.1	−11.3	3.64	3.16	−0.47	3,471	2,715	−756	152	128	−24	9.0	17.1	+8.1
P	NS	NS	NS	<0.05	NS	<0.05	<0.01	NS	NS	NS	NS	<0.05	<0.05	NS	<0.01	<0.05	<0.05	NS
Exercise																		
No drugs	199	204	+5	33.2	26.4	−6.7	6.32	5.15	−1.17	5,192	4,335	−857	178	151	−27	7.1	10.8	+3.8
Mannitol	176	184	+7	34.8	27.1	−7.5	6.01	4.83	−1.18	5,193	3,955	−1,238	173	147	−26	6.7	18.8	+12.1
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

For symbols and abbreviations see footnote to Table 2.

statistically significant changes due to mannitol either prior to or during occlusions (Table 4).

DISCUSSION

Our results demonstrated that brief occlusion of the left circumflex coronary artery resulted in changes that were remarkably similar, whether a dog was standing quietly or running on a treadmill. These included decreases in dP/dt max (−20% standing or running), stroke volume (−25% standing, −23% running), and left ventricular systolic pressure (−13% standing, −21% running), and a rise in left ventricular end-diastolic pressure (+4.4 mmHg standing, +6.3 mmHg running). The exception was a greater degree of tachycardia when animals were at rest. These results were somewhat unexpected in view of the considerably higher myocardial oxygen consumption known to occur during exercise. There are at least two possible explanations for such surprisingly good ventricular function in the face of ischemia during exercise. Increased flow due to vasodilation in collateral vascular pathways during exercise may prevent impairment of the ratio of myocardial oxygen supply to demand in the region normally

served by the occluded artery (11). Another possibility is that the higher levels of generalized sympathetic activity associated with exercise sufficiently enhance function in normally perfused regions to offset impaired function in the ischemic region.

There is some disparity between our results in the studies with occlusion of the left circumflex coronary artery and the results of Vatner et al. (14), in which stenosis without occlusion of the main left coronary produced only slight decrements in left ventricular performance at rest but profound deterioration during exercise. Distal vasodilation can often maintain flow through a stenotic coronary artery at nearly normal levels under resting conditions (5), but when myocardial oxygen demands increase with exercise, flow through the stenosis may not increase further. In contrast, total occlusion of any coronary artery is likely to result in a region of distal ischemia at rest (2–4, 6, 10), as well as during exercise. Since obstruction of the main left coronary may cause global ischemia of the left ventricle, whereas distal occlusions permit normal perfusion in some portions of the ventricle, the extent to which ischemia-induced decrements in integrated left ventricular performance can be exacerbated by exercise stress may depend upon the percentage of the left ventricle that is not ischemic. In addition, the possibility that collateral flow can increase during exercise in regional ischemia cannot be excluded.

The major difference in hemodynamics between the effects of regional myocardial ischemia at rest and during running was in heart rate. Although stroke volume fell in both settings, cardiac output was maintained at preocclusion levels at rest as a result of the heart rate increase. During exercise occlusions, cardiac output fell because heart rate did not increase significantly. A previous study has concluded that tachycardia due to temporary left circumflex coronary artery occlusion in the resting, conscious dog is reflex in origin (13). This reflex is initiated by receptors near the heart, involves both vagal and sympathetic efferent pathways, and is modulated by arterial baroreceptor activity (13). During exercise, sympathetic tone is high and vagal tone is substantially reduced (7, 8). These changes may be so marked that the capability of the reflex mechanism to induce further sympathetic stimulation or vagal withdrawal via the efferent pathways is limited. Alternatively, as appears to be the case with baroreceptor reflex control of heart rate (12), the sensitivity of the reflex decreases, either through diminished receptor sensitivity or central nervous system changes.

In the dosage used in this study, propranolol effectively competes with endogenous catecholamines for beta-adrenergic receptor sites (8). As has been reported previously (10), propranolol had little effect on the inotropic state during resting conditions either before or during occlusions. On the basis of this finding, it would appear that increases in sympathetic tone may be small during occlusions at rest. During exercise, when sympathetic tone is markedly augmented, pretreatment with propranolol significantly limited increases in heart rate, cardiac output, and rate of force development (dP/d*t* max). However, exercise occlusions resulted in changes similar in magnitude to those that occurred without propranolol. Thus, since beta-adrenergic blockade depressed cardiac performance during exercise, and changes due to ischemia were the same as those seen without propranolol, it is likely that propranolol primarily influenced function in normal rather than ischemic regions. This is compatible with the concept that, when autonomic control is intact, the ameliorative effect of catecholamines on the nonischemic myocardium overcomes the deleterious effects of increased myocardial oxygen demand in the ischemic region.

Ouabain increased the rate of force development at rest prior to occlusion. The rate of force development and peak systolic pressure were higher during resting occlusion than those that occurred without drugs, although the changes due to occlusion did not differ. Ouabain did not alter dP/d*t* max during exercise either before or during ischemia. The lack of effect of ouabain during exercise may mean that stimulation by catecholamines increased myocardial contractile force in normally perfused regions to such high levels that inotropic agents can add little additional benefit.

Mannitol also increased dP/d*t* max prior to occlusion as has been reported previously (1). However, mannitol has no effect on dP/d*t* max during rest occlusions or during exercise states. Mannitol increased left ventricular end-diastolic pressure during ischemia at rest. Whether this reflects increased stiffness of the left ventricle or enhanced venous return due to expansion of blood volume or other factors cannot be determined from our data.

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REFERENCES

1. Atkins, J. M., K. Wildenthal, and L. D. Horwitz. Cardiovascular responses to hyperosmotic mannitol in anesthetized and conscious dogs. *Am. J. Physiol.* 225: 132–137, 1973.
2. Bishop, V. S., R. L. Kaspar, G. E. Barnes, and M. B. Kardon. Left ventricular function during acute regional myocardial ischemia in the conscious dog. *J. Appl. Physiol.* 37: 785–792, 1974.
3. Dwyer, E. W. Left ventricular pressure-volume alteration and regional disorders of contractions during ischemia induced by atrial pacing. *Circulation* 42: 1111–1122, 1970.
4. Enright, L. P., H. Hannah, and R. L. Reiss. Effects of acute regional myocardial ischemia in left ventricular function in dogs. *Circulation Res.* 26: 307–315, 1970.
5. Gould, K. L., K. Lipscomb, and G. W. Hamilton. Physiologic basis for assessing critical coronary stenosis. *Am. J. Cardiol.* 33: 87–94, 1974.
6. Hood, W. B., Jr., V. H. Covelli, W. H. Abelmann, and J. C. Norman. Persistence of contractile behavior in acutely ischemic myocardium. *Cardiovascular Res.* 3: 249–260, 1969.

7. HORWITZ, L. D., J. M. ATKINS, AND S. J. LESHIN. Role of the Frank-Starling mechanism in exercise. *Circulation Res.* 31: 868–874, 1972.
8. HORWITZ, L. D., J. M. ATKINS, AND S. J. LESHIN. Effects of beta-adrenergic blockade on left ventricular function in exercise. *Am. J. Physiol.* 227: 839–842, 1974.
9. HORWITZ, L. D., AND V. S. BISHOP. Left ventricular pressure-dimension relationships in the conscious dog. *Cardiovascular Res.* 6: 163–171, 1972.
10. KASPAR, R. L., G. E. BARNES, D. F. PETERSON, AND V. S. BISHOP. Specificity of autonomic influences on cardiac responses during myocardial ischemia. *J. Appl. Physiol.* 38: 485–490, 1975.
11. LAMBERT, P. R., D. S. HESS, AND R. J. BACHE. Effect of exercise on perfusion of collateral-dependent myocardium in dogs with chronic coronary artery occlusion. *J. Clin. Invest.* 59: 1–7, 1977.
12. MCRITCHIE, R. J., S. F. VATNER, D. BAELTCHER, G. R. HEYNDRICKZ, T. A. PATRICK, AND E. BRAUNWALD. Role of arterial baroreceptors in mediating cardiovascular response to exercise. *Am. J. Physiol.* 230: 85–89, 1976.
13. PETERSON, D. F., R. L. KASPAR, AND V. S. BISHOP. Reflex tachycardia due to temporary coronary occlusion in the conscious dog. *Circulation Res.* 32: 652–658, 1973.
14. VATNER, S. F., R. J. MCRITCHIE, P. R. MAROKO, T. A. PATRICK, AND E. BRAUNWALD. Effects of catecholamines, exercise and nitroglycerin on the normal and ischemic myocardium in conscious dogs. *J. Clin. Invest.* 54: 563–575, 1974.

Anti-G suit effect on cardiovascular dynamic changes due to $+G_z$ stress

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PETERSON, D. FRED, VERNON S. BISHOP, AND HOWARD H. ERICKSON. *Anti-G suit effect on cardiovascular dynamic changes due to $+G_z$ stress*. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 43(5): 765–769, 1977. – Lightly anesthetized dogs underwent 1-min exposure to $+G_z$ acceleration without and with a bladder-type anti-G suit. Prior chronic instrumentation permitted thorough evaluation of cardiac dynamics. During +3 G_z acceleration all recorded dynamic variables were lowered and transient tachycardia occurred. After acceleration ceased, all pressures and dP/dt exceeded control levels. Inflation of the anti-G suit during +3 G_z eliminated the dramatic effects observed during and after acceleration stress. During +6 G_z with the anti-G suit inflated, arterial pressure and dP/dt were maintained whereas left ventricular end-diastolic pressure and total peripheral resistance were much elevated and heart rate was lower. At the onset of G stress, internal diameter of the heart always fell transiently. Otherwise, diameter was not significantly affected by any of the experimental conditions. The results suggest that the anti-G suit maintains perfusion pressure at high sustained G; however, with the anti-G suit inflated at +6 G_z, central venous pressure is dramatically elevated and heart rate depressed. Thus, beneficial effects which provide tolerance to high G are accompanied by potentially detrimental effects.

centrifugation; acceleration stress; dogs

LAMBERT AND WOOD (10) confirmed that the limiting factor in $+G_z$ stress tolerance in humans was blackout and unconsciousness due to compromised perfusion pressure to the central nervous system. Tolerance can be extended by performance of an M-1 (straining) maneuver (16) and even further extended by wearing an anti-G suit (5, 7).

Since the primary concern during $+G_z$ acceleration has been that pilots could adequately operate an aircraft, little in-depth evaluation of cardiovascular effects have been investigated until very recently. In conscious man, studies had determined that heart rate usually rose and blood pressure fell (12). Lightly anesthetized dogs exhibit the same responses (3, 8, 13). ECG abnormalities occurred but did not persist long after cessation of acceleration (12, 14, 15). Similar findings have been observed in experimental animals (3, 4), but in addition, pathological changes have been observed to occur in the myocardium of miniature swine (6).

Chronic instrumentation has made possible much more sophisticated evaluation of cardiovascular responses in experimental animals (3, 8, 13). During $+G_z$ acceleration of 2 G or greater, cardiovascular function is depressed, and the severity of depression is directly related to the magnitude of acceleration (13). Additionally, the immediate postacceleration period is characterized by elevated arterial pressure, left ventricular end-diastolic pressure, and peripheral resistance; and these changes persist for several minutes.

Although subjective tolerance to acceleration stress is improved by wearing an anti-G suit, the nature and magnitude of changes in many cardiovascular variables is not known.

The present study was designed to determine the degree and nature of cardiovascular protection provided by an abdominal bladder-type anti-G suit during and after $+G_z$ acceleration. Additionally, since recent studies have demonstrated subendocardial damage associated with acceleration stress, changes in left ventricular internal diameter were investigated.

METHODS

Sixteen mongrel dogs (10–20 kg) were anesthetized with halothane and chronically instrumented under sterile surgical conditions. A left thoracotomy through the fifth intercostal space exposed the heart and great vessels. The pericardium was opened, and through a stab incision, a solid-state pressure transducer (model P18, Konigsberg Instruments) was placed on the endocardial surface of the left ventricle in eight dogs to measure left ventricular pressure. An electromagnetic flow probe (Zepeda Instruments) was placed around the ascending aorta to measure cardiac output in six of these dogs, and an 18-gauge polyvinyl catheter was placed in the left atrium to measure pressure. In 10 dogs, two sonomicrometer transducers were implanted across the greatest transverse diameter of the left ventricular chamber, using techniques previously described (1, 2). ECG electrodes were sutured inside the chest. Lead wires for the implanted instrumentation were exteriorized at the back of the neck. Two weeks or longer were allowed for recovery. During this time, the health of each animal was monitored daily.

Prior to experimentation, animals were anesthetized

with alpha-chloralose to a light plane of surgical anesthesia. A precalibrated, solid-state pressure catheter (no. 5F, model PC-350, Millar Instruments) was then passed retrograde via the femoral artery into the left ventricle to calibrate the left ventricular pressure transducer already in place. After calibration, the catheter was withdrawn to lie in the root of the aorta. Each dog was restrained on its back ($+G_x$) in a fiberglass animal couch which was bolted to the animal end of the USAFSAM centrifuge. (The length of the arm for animal experiments is 4 m.) The dogs were positioned to receive $+G_z$ inertial forces as the centrifuge rotated, and an abdominal bladder-type anti-G suit was placed beneath each animal's back but not attached. Once the animal was properly positioned, a 15- to 30-min pretest period was allowed to establish resting levels of recorded variables. Experiments were begun about 1 h after initial administration of anesthesia. At this time, slight spontaneous body movements were often observed.

Each animal was initially exposed to a gravitoinertial force of $+2$ G_z for 1 min. After the animal had recovered from this trial, the anti-G suit was attached around the abdomen, and the animal was exposed to $+2$ and $+3$ G_z acceleration without inflation of the suit. Finally, the anti-G suit was set to activate automatically at 2.2 G so that pressure within the suit increased at 1.5 lb/in.^{2}·G. Although the surface of the body influenced by the suit was limited to the abdomen, previous studies in humans have indicated that 75% of the relaxed increase in G_z tolerance is accomplished with such a suit (5, 17). Trials of $+3$ and $+6$ G_z were then carried out. A 5- to 15-min recovery was allowed after each trial before starting another trial. Onset to peak G was considered rapid (1.0 G/s), and peak acceleration was always maintained for 1 min.

Responses were recorded on a Mark 200 Brush strip-chart recorder and simultaneously on a model 4742 Sangamo magnetic tape recorder for later analysis on an Electronics Associates 680 analog computer.

Total peripheral resistance (TPR) was calculated by the computer as mean aortic root pressure (AP) minus left ventricular end-diastolic (LVEDP) divided by mean aortic flow (AF), that is

$$TPR = \frac{AP(Torr) - LVEDP(Torr)}{AF(ml/min)}$$

In some cases, calibration of mean aortic flow was not possible for technical reasons; hence, our results are all expressed in percent changes in total peripheral resistance. This permitted us to include results in which mean flow values were not expressed in absolute units. A paired *t*-test was used to determine whether or not differences between trials were significant.

RESULTS

Responses to $+G_z$ acceleration in a single animal are illustrated in Fig. 1. Without the anti-G suit, changes were similar to those previously reported (13). Qualitatively, responses to $+2$ and $+3$ G_z were the same although, as previously reported (13), the magnitude of change was greater due to $+3$ G_z. Inflation of the anti-G suit during $+3$ G_z eliminated the depressed responses and, rather, tended to elevate most variables slightly. During $+6$ G_z acceleration, changes were less consistent. Pressures were usually elevated whereas cardiac function was depressed, as reflected by dP/dt, heart rate, and left ventricular diameters. Placement of the uninflated anti-G suit around the abdomen did not affect the responses to $+2$ G_z acceleration.

Arterial pressure. During $+3$ G_z acceleration, average mean aortic arch pressure fell rapidly and then gradually recovered toward control (Fig. 2; Table 1). In the postacceleration period, a dramatic increase in blood pressure was observed which did not return to 50% of control until 3.2 ± 0.6 min after acceleration ceased. During both $+3$ and $+6$ G_z acceleration, with the anti-G suit inflated, average mean arterial pressure remained above control after a small initial fall in mean pressure. Upon termination of $+3$ G_z with the suit, aortic pressure fell slightly below control then recovered rapidly. After $+6$ G_z, an elevation in pressure was observed which recovered slowly, much as in the unprotected trials (Table 1; Fig. 2). In every instance, peak left ventricular systolic pressure underwent the same qualitative changes as aortic arch pressure (Fig. 1).

Heart rate. Heart rate rose from 99 to 179 beats/min

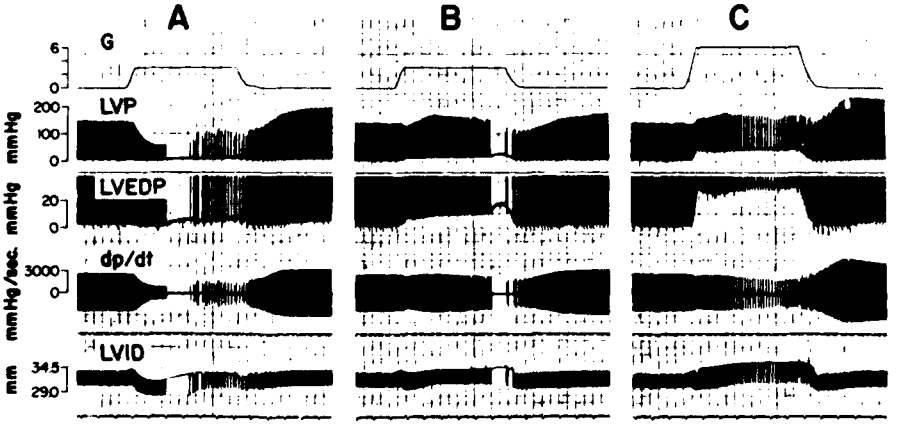

FIG. 1. Effects of $+G_z$ acceleration with and without an anti-G suit. Responses to $+3$ G_z without anti-G suit are illustrated in *panel A*. Responses with suit inflating at 2.2 G are illustrated in *panel B* ($+3$ G_z) and *panel C* ($+6$ G_z). In each panel, top trace represents the G profile; LVP = left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; dP/dt = first derivative of LVP; LVID = left ventricular internal diameter. Bottom trace is time in seconds.

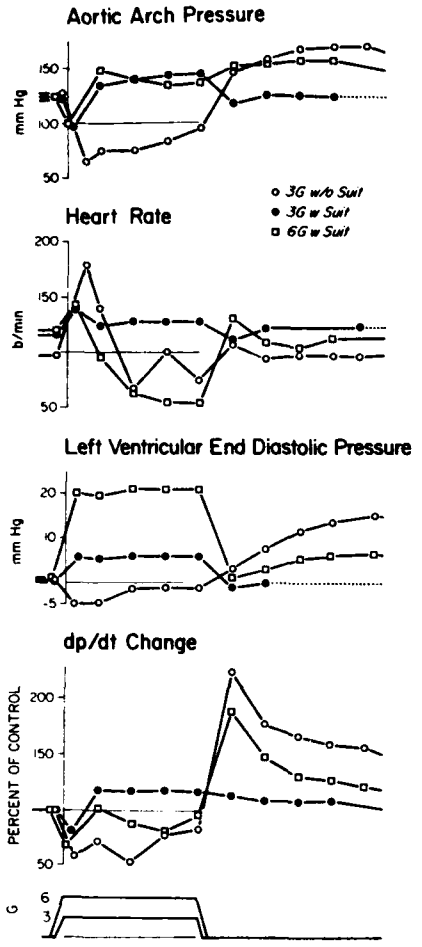

FIG. 2. Average responses of 8 dogs to acceleration stress. After onset of acceleration, initial peak response is shown. Thereafter, average values were determined every 15 s from initial point of peak acceleration. Duration of maximum acceleration (60 s) is indicated by horizontal bar at bottom of figure. Control values indicate point of onset of acceleration and ordinate indicates point at which maximum acceleration was reached.

($P < 0.001$) at 13.4 s after onset of acceleration. The time to onset and peak responses for mean arterial pressure were significantly earlier than for heart rate ($P < 0.01$), suggesting that the tachycardia occurred in response to the fall in blood pressure. Heart rate fell rapidly after reaching the peak and, in six of eight animals, was below control during most of the acceleration period, although heart rate was usually erratic and there were large differences between animals. Brief periods of asystole were not uncommon, and two dogs experienced sustained tachycardia. After acceleration ceased, there was no consistent heart rate pattern. Responses ranged from −34 to +67 beats/min from control. Activation of the anti-G suit during +3 G_z eliminated the erratic heart rate pattern observed in most dogs without the suit. The exception to this observation is seen in Fig. 1, in which a brief period of asystole occurred toward the end of acceleration. A transient rise in heart rate was observed 11.5 s after onset of +6 G_z acceleration with the suit, followed by a progressive fall in heart rate to 54 beats/min at 60 s. All values during G after 15 s were significantly less than control ($P < 0.001$), Fig. 2.

TABLE 1. *Peak effects and time to effects during and after +G_z acceleration*

	Pre G Control	During G		Post G	
		Peak effect	Time to peak	Peak effect	Time to 50% recovery
	Torr	*Torr*	*s*	*Torr*	*min*
AP					
3 G w/o	128 ± 7	67 ± 13*	11.5 ± 1.5	168 ± 12†	3.2 ± 0.6
3 G w	122 ± 9	98 ± 9*	5.9 ± 0.4	117 ± 11	0.6 ± 0.2
6 G w	125 ± 9	100 ± 9‡	6.1 ± 0.7	153 ± 6‡	2.5 ± 0.6
	beats/min	*beats/min*	*s*	*beats/min*	*min*
HR					
3 G w/o	99 ± 8	179 ± 15*	13.4 ± 1.2	94 ± 9	1.7 ± 0.5
3 G w	112 ± 13	140 ± 14	9.0 ± 0.8	108 ± 7	0.5 ± 0.1
6 G w	116 ± 12	143 ± 15	11.5 ± 1.5	126 ± 14	2.1 ± 0.6
	Torr	*Torr*	*s*	*Torr*	*min*
LVEDP					
3 G w/o	1.4 ± 0.7	−5.0 ± 1.8‡	6.9 ± 0.8	15.0 ± 3.3†	2.4 ± 0.4
3 G w	1.0 ± 0.8	6.3 ± 1.1*	6.1 ± 0.4	1.3 ± 0.8	0.5 ± 0.1
6 G w	1.9 ± 0.8	20.7 ± 2.5*	7.6 ± 0.3	4.9 ± 1.0†	1.4 ± 0.3
	%	%	*s*	%	*min*
dP/dt					
3 G w/o	100	58 ± 7*	8.0 ± 0.9	216 ± 47‡	1.3 ± 0.5
3 G w	100	82 ± 7‡	6.3 ± 0.6	112 ± 11	0.5 ± 0.1
6 G w	100	68 ± 9†	6.2 ± 0.7	191 ± 24†	0.9 ± 0.2

Values represent mean ± SE for 8 dogs; w/o, without inflation of the anti-G suit; w, with inflated suit. * $P < 0.001$. † $P < 0.01$. ‡ $P < 0.05$.

Left ventricular end-diastolic pressure (LVEDP). Without the anti-G suit, LVEDP fell significantly (−5.0 Torr) but recovered before the end of acceleration. A large increase was observed after G stress (15.0 Torr), and 50% recovery to control was not reached until 2.4 min after the end of acceleration (Table 1). The anti-G suit profoundly altered these responses. LVEDP was elevated throughout acceleration, and the effect due to +6 G_z (20.7 Torr) was much greater than that due to +3 G_z (6.3 Torr). After acceleration at +3 G_z with the suit, LVEDP was 50% recovered by 30 s. After +6 G_z, LVEDP fell transiently and then rose significantly above control (4.9 Torr; $P < 0.01$) (Fig. 2; Table 1).

Time derivative of left ventricular pressure. Without the anti-G suit, changes in left ventricular peak dP/dt were also dramatic. During +3 G_z, dP/dt fell and, in one animal, remained depressed (Fig. 2). Average maximum fall was −45%. In the postacceleration period, dP/dt rose immediately to an average maximum increase of 116% above control at 15 s and then gradually returned to control. Fifty percent recovery to control was complete at 1.3 min after the end of acceleration. The anti-G suit eliminated dramatic changes in dP/dt at +3 G_z. After an initial transient drop, dP/dt rose to about 15% above control; and this proved to be a significant elevation at 30, 45, and 60 s of +3 G_z. After acceleration, average dP/dt was not significantly elevated.

During +6 G_z with the suit, all animals experienced an initial fall (−32%; $P < 0.01$), after which no consistent changes could be demonstrated during G. After G, dP/dt rose to 91% above control ($P < 0.01$) and recovered rapidly to 50% of control (0.9 min).

Aortic flow. Both stroke volume and aortic flow were depressed under all conditions of acceleration stress. In the cases of +3 G_z without (−66%) and +6 G_z with the

suit (−55%), magnitude of mean flow depression was greater than during +3 G_z with the suit inflated (−27%). Flow remained depressed throughout acceleration and, immediately upon stopping the centrifuge, rose back toward control under all three conditions. After +3 G_z without the suit, however, this recovery was transient. Flow was depressed again by 23% ($P < 0.01$). On the other hand, after +3 and +6 G_z with the suit, no significant deviation from control was demonstrated.

Peripheral resistance. Calculation of peripheral resistance at regular intervals was complicated by the periods of extremely erratic cardiac output at low heart rates as well as the occasional periods of observed asystole. For this reason peripheral resistance was sampled during periods of regular cardiac output. In all cases, calculated TPR was elevated during acceleration. During +3 G_z with the suit, the elevation was less than in the other cases. The maximum value was 60% above control. During +3 G_z without the suit, average maximum TPR was 124% above control. During +6 G_z with the suit inflated, average maximum TPR was 217% above control. After 3 G_z without the suit, TPR fell transiently toward control, then climbed significantly above control (58 ± 21; $P < 0.05$) at 1 min after acceleration ceased. After +3 G_z with the suit inflated, TPR returned rapidly to control. After +6 G_z with the suit inflated, TPR returned gradually to control but did remain significantly elevated at 1 min post-G (+37 ± 15; $P < 0.05$).

Left ventricular internal diameter. Immediately after the onset of acceleration, the left ventricular internal diameter (LVID) began to get smaller. During +3 G_z without the suit, at the time when arterial pressure was lowest (64 Torr for this group), systolic size of the heart was significantly reduced by 2.2 mm ($P < 0.01$) to 28.3 mm. In no case did the left ventricle approach collapse. The average diameter in diastole fell 0.8 mm, but the change was not significant. As arterial pressure recovered, LVID's got larger and no other significant changes in left ventricular size were observed during or following +G_z acceleration. It was suspected that the magnitude of change in diameter size might be related to either arterial pressure or heart rate. Analysis of variance showed no significant relationship in either case. Even in animals whose arterial pressure fell dramatically, diameter size was not significantly altered. In five dogs whose left ventricular systolic pressure fell below 60 Torr (avg, 49 Torr), end-systolic diameter fell by only 2.4 mm. In three dogs whose heart rates rose 50 beats/min or more during acceleration (avg, 101 beats/min), end-systolic diameter fell by 4.7 mm to an average of 25.3 mm.

Both increases and decreases in internal diameter were observed with the suit activated. Responses in four dogs were similar to those illustrated in Fig. 1. In these experiments LVID got small during unprotected acceleration and larger if the anti-G suit was inflated. In the other six dogs no general pattern could be identified. Thus, there were no consistent changes in internal diameter during activation of the anti-G suit which could be demonstrated statistically.

DISCUSSION

Results of this study demonstrated that activation of an abdominal bladder-type anti-G suit reversed the deleterious cardiovascular effects due to +3 G_z stress. Elevation of aortic arch pressure above control suggests an especially beneficial effect in preventing blackout and unconsciousness which occurs in man at low central perfusion pressures. Most other untoward effects of acceleration were not observed to occur at +3 G_z with the suit, except at the onset of acceleration. A transient fall in arterial pressure, left ventricular pressure, and dP/dt were observed. Since the suit was not activated until 2.2 G was reached, these changes were undoubtedly due to suddenly reduced afterload associated with onset of increased gravitoinertial forces. The slightly delayed tachycardia indicates a baroreflex response to the pressure fall. After activation of the anti-G suit, both elevation of arterial pressure and left ventricular end-diastolic pressure might be accounted for by a shift in circulating body fluids. Reduction of venous pooling in the abdomen would increase venous return and increase pulmonary blood volume (9), whereas the anti-G-suit-induced increase in total peripheral resistance would tend to impede cardiac output. The likelihood that left ventricular end-diastolic pressures do mirror the filling pressure of the left heart is supported by the results in Fig. 1 indicating that end-diastolic heart size was directly related to LVEDP in this animal. On the other hand, as previously reported (9), pressure on the abdomen may displace the diaphragm and increase intrathoracic pressure, thus directly influencing thoracic vascular pressures. Near-normal heart rate suggested that neither direct stress nor indirect reflex influences were dramatically altering heart function. In the post-G period all variables approached control rapidly, indicating lack of sustained adverse cardiovascular effects at +3 G_z with the anti-G suit inflated. Thus, the prolonged elevation of arterial pressure and LVEDP observed here and previously (13) in the post-G period were completely eliminated.

At +6 G_z, partial protection from adverse cardiovascular effects was obtained by suit inflation. Most changes were intermediate between those observed at +3 G_z with and without the suit. Average aortic arch pressure was maintained above control, and although it was not a significant elevation, this is in marked contrast to trials without the anti-G suit. In a previous study, due to poor recovery from +3 or +4 G_z, only 4 of 14 dogs could be tested at +5 G_z without the anti-G suit (13). In a similar study hypotension, bradycardia, and a profound decrease in coronary blood flow were reported during exposure to +5 or +6 G_z stress (8). Thus, maintenance of perfusion pressure to the coronary and CNS vasculature was dramatically improved by the suit during high acceleration stress.

Although arterial pressure was maintained, significant decrements in other cardiovascular indices were observed. Heart rate was down, and this could not be attributed to reflex bradycardia. Aortic flow was dramatically down, which could perhaps be related to both depressed heart rate and limited circulating blood vol-

ume. The most consistent observation was the dramatic elevation in LVEDP. This suggests that during high sustained +G_z with an anti-G suit, elevated intrathoracic pressures are probably a necessary compromise in order to maintain aortic arch perfusion pressure. On the other hand, in the post-6-G period, the overshoot changes in aortic pressure, dP/dt, and LVEDP were not as great nor did they persist as long as in the unprotected trials at +3 G_z.

Our results indicated that although lightly anesthetized dogs did not tolerate +3 G_z acceleration well without the protection of an anti-G suit, changes in left ventricular internal diameter were not as dramatic as were changes in other variables. Numerous studies have indicated that pathological changes in cardiac muscle are associated with high sustained acceleration stress (6, 11). In the present study, we considered the possibility that the subendocardial hemorrhage reported by MacKenzie et al. (11) might be due to striking of the internal walls of the left ventricle during acceleration. No evidence for this possibility was obtained. Although peak left ventricular pressure fell as low as 30 Torr in one dog, and heart rate rose to 280 beats/min in another, in no animal was the left ventricle observed to get smaller than 15.5 mm across its greatest diameter during end-systole. In an earlier study, severity of the pathological findings was related to *a*) level and duration of G exposure, *b*) heart rate, and *c*) catecholamine activity (6). It does not appear that these changes can induce striking of the internal walls of the left ventricle during stresses similar to those in this study. Changes during inflation of the anti-G suit were even less remarkable. In those cases no significant changes in left ventricular internal diameter could be demonstrated.

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REFERENCES

1. BISHOP, V. S., AND L. D. HORWITZ. Effects of altered autonomic control of left ventricular function in conscious dogs. *Am. J. Physiol.* 221: 1278–1282, 1971.
2. BISHOP, V. S., L. D. HORWITZ, H. L. STONE, H. F. STEGALL, AND E. J. ENGELKEN. Left ventricular internal diameter and cardiac function in conscious dogs. *J. Appl. Physiol.* 37: 785–792, 1969.
3. BURNS, J. W. Influence of ethanol on cardiovascular tolerance to +G_z acceleration. *Aerospace Med.* 45: 19–28, 1974.
4. BURTON, R. R. Positive (+G_z) acceleration tolerances of the miniature swine: application as a human analog. *Aerospace Med.* 44: 294–298, 1973.
5. BURTON, R. R., AND R. W. KRUTZ, JR. G-tolerance and protection with anti-G suit concepts. *Aviation Space Environ. Med.* 46: 119–124, 1975.
6. BURTON, R. R., AND W. F. MACKENZIE. Cardiac pathology associated with high sustained +G_z. I. Subendocardial hemorrhage. *Aviation Space Environ. Med.* 47: 711–717, 1976.
7. BURTON, R. R., M. J. PARKHURST, AND S. D. LEVERETT, JR. +G_z protection afforded by standard and preacceleration inflations of the bladder and capstan-type G-suits. *Aerospace Med.* 44: 488–494, 1973.
8. ERICKSON, H. H., H. SANDLER, AND H. L. STONE. Cardiovascular function during sustained +G_z stress. *Aviation Space Environ. Med.* 47: 750–758, 1976.
9. HOWARD, P. The physiology of positive acceleration. In: *A Textbook of Aviation Physiology*, edited by J. A. Gillies. New York: Pergamon, 1965, chapt. 23, p. 615–618.
10. LAMBERT, E. H., AND E. H. WOOD. The problem of blackout and unconsciousness in aviators. *Med. Clin. N. Am.* 30: 833–844, 1946.
11. MACKENZIE, W. F., R. R. BURTON, AND W. I. BUTCHER. Cardiac pathology associated with high sustained +G_z. II. Stress cardiomyopathy. *Aviation Space Environ. Med.* 47: 718–725, 1976.
12. PARKHURST, M. J., S. D. LEVERETT, JR., AND S. J. SHUBROOKS, JR. Human tolerance to high, sustained +G_z acceleration. *Aerospace Med.* 43: 708–712, 1972.
13. PETERSON, D. F., V. S. BISHOP, AND H. H. ERICKSON. Cardiovascular changes during and following 1-min exposure to +G_z stress. *Aviation Space Environ. Med.* 46: 775–779, 1975.
14. SHUBROOKS, S. J., JR. Changes in cardiac rhythm during sustained high levels of positive (+G_z) acceleration. *Aerospace Med.* 43: 1200–1206, 1972.
15. SHUBROOKS, S. J., JR. Positive pressure breathing as a protective technique during +G_z acceleration. *J. Appl. Physiol.* 35: 294–298, 1973.
16. SHUBROOKS, S. J., JR., AND S. D. LEVERETT, JR. Effect of the valsalva maneuver on tolerance to +G_z acceleration. *J. Appl. Physiol.* 34: 460–466, 1973.
17. WOOD, E. H., AND E. H. LAMBERT. Some factors which influence the protection afforded by pneumatic anti-G suits. *Aerospace Med.* 23: 218–228, 1952.

Influences of Selective Cardiac Denervation on Coronary Reactive Hyperemia In Conscious Dogs

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Abstract

Mongrel dogs were chronically instrumented to measure left circumflex coronary flow, arterial pressure, left atrial pressure, ECG, heart rate and in some cases left ventricular pressure or cardiac output. A cuff type occluder was placed distal to the coronary flow probe. Total occlusion of the left circumflex coronary artery for one minute in unsedated, resting dogs produced reactive hyperemia with an average replacement/deficit ratio of 2.68/1. In 10 dogs sympathetic influences were investigated by total, chronic cardiac sympathectomy. Surgical section of all ansae subclavia reduced responses from 2.76/1 to 1.71/1 ($P < .001$). Left sympathectomy alone had no effect on the replacement/deficit ratio whereas selective right sympathectomy reduced it from 2.61/1 to 1.67/1. Pharmacological blockade was used to determine beta-receptor involvement in the responses. In 9 intact dogs practolol (10 mg/kg) reduced the reactive hyperemia ratio by 11% ($P < .05$). Propranolol (1 mg/kg) further reduced this ratio by 30% ($P < .001$). Our results indicate that sympathetic beta influences work primarily through the right cardiac sympathetic nerves. Also, the magnitude of the response appears to be due, in part, to increased metabolic activity associated with myocardial β_1 receptors as well as vasodilation through β_2 receptors.

KEY WORDS: reflex circumflex coronary artery propranolol practolol cardiac sympathectomy

Numerous studies have indicated that coronary blood flow can be influenced by both the parasympathetic and sympathetic nervous systems (1-6). Direct efferent stimulation of the vagus nerve produces coronary vasodilation independent of chronotropic or inotropic effects suggesting the potential for a parasympathetic component in the regulation of coronary blood flow (4). More recently, reflex vasodilation via vagal efferents has been demonstrated to accompany injection of veratrum alkaloids into the coronary circulation (7). Sympathetic involvement in coronary blood flow regulation is more complex. Evidence indicates that direct neural control of coronary resistance is partially mediated through sympathetic alpha receptors located in the vessel walls (3,5). However, vasodilation is observed during cardiac sympathetic nerve stimulation. In general this dilation has been assumed to be secondary to the increased metabolic requirements associated with the inotropic and chronotropic responses to myocardial β_1-receptor stimulation (6). Thus, in the intact heart, the regulation of oxygen delivery through changes in coronary resistance appears to be dominated by changes in local oxygen requirements. However, there is some evidence for an intrinsic adrenergic vasodilator mechanism (2). Furthermore, in the cat, stimulation of coronary sC fibers has been shown to decrease coronary resistance independent of changes in the chronotropic or inotropic state of the myocardium (8).

Brief occlusion of a coronary artery results in a reactive hyperemia response in which the flow far exceeds the apparent deficit resulting during the period of occlusion. This response has been linked to both myogenic relaxation of the vessels (9) and to the myocardial metabolic requirements (1,10,11). Whether or not the nervous system is involved in coronary reactive hyperemia has not been determined.

In the present study, we have investigated the involvement of the cardiac sympathetic neural reflexes in modulating the magnitude of the coronary reactive hyperemia in the conscious dog. The magnitude of the hyperemia was determined following one minute occlusion of the left circumflex coronary artery. Control responses were compared to those after both partial and total cardiac sympathectomy as well as after selective beta adrenergic blockade and after atropine.

Methods

Chronic Surgery.

Twenty-six mongrel dogs weighing between 10-25 kg were chronically instrumented using sterile surgical techniques under halothane (Fluothane, Ayerst) anesthesia. A left thoracotomy was performed through the fifth intercostal space exposing the heart and great vessels. The pericardium was opened and a longitudinal incision made in the connective tissue over the left circumflex artery just beyond its origin. A 6-10 mm length was lifted away from surrounding tissue and a balloon

type polyethylene catheter cuff, similar to that previously reported (12) was placed around the vessel. The catheter was then sutured to the wall of the ventricle, thus, immobilizing it. A saline filled syringe was used to verify occlusion. Both the volume and pressure required for occlusion were determined. An electromagnetic flow probe (Zepeda) was placed around the left circumflex coronary artery proximal to the occluder in all dogs for measurement of coronary flow. During implantation of the occluder and flow probe, special care was taken to avoid damage to cardiac nerves. This was accomplished by instrumenting only those dogs in which the circumflex artery was located superficially and could be lifted away from underlying tissue with minimal tissue trauma. Undoubtedly, some damage was done to innervation of this segment of the artery, however, the main nerve trunk was carefully avoided.

An 18-gauge polyvinyl catheter was placed in the left atrial appendage for measurement of pressure or infusion of drugs. A second catheter was placed directly into the aortic arch at the time of thoracotomy or into a carotid artery through a cervical incision several days later. During the initial surgery a loop of surgical suture (Tevdek) was placed around the left ansae subclavia as they emerged from the stellate ganglion, thus encircling the left cardiac sympathetic nerves (13). In 8 dogs an electromagnetic flow probe was placed around the ascending aorta and in 16 a solid state pressure transducer placed in the left ventricle via a stab incision in the

myocardium. All catheters, leads, thread, and the occlusive device were exteriorized at the back of the neck. Approximately two weeks were allowed for recovery and, at the time coronary occlusions were performed, body temperature and ECG were normal.

Experimental Protocol.

ECG, blood pressure, heart rate, left ventricular pressure, aortic flow and left circumflex coronary flow were recorded simultaneously on a Beckman Type R Dynograph and Ampex tape recorder using appropriate transducers, couplers, and amplifiers. Coronary occlusions were performed by inflating the coronary cuff with saline and occlusions were maintained for one minute. In our experiments, one minute occlusions of the left circumflex coronary artery only rarely produced extrasystolic beats.

Unless otherwise indicated, experiments were performed while each dog was conscious, lying on its right side unrestrained. After control values had been obtained, responses to selective cardiac sympathectomy were studied. Sterile surgery was performed a second time in order to denervate the right cardiac sympathetic innervation. In 3 dogs, only the left ansae subclavia were sectioned. In 11 dogs, both right and left were sectioned. However, in 5 of these dogs the left side remained intact until responses could be studied after selective right-side section. In all cases the dogs were anesthetized prior to pulling the thread which encircled the left ansae subclavia. Post morteum examination verified that all branches emerging from the stellate ganglia were severed on both the right and

left sides. On each experimental dog, three responses were obtained at 5-10 minute intervals. The average of each three responses was considered the mean response for that series and represented a single response for statistical purposes.

Control responses as well as responses after practolol (10 mg/kg), propranolol (1 mg/kg) and atropine (0.1 mg/kg) were obtained in dogs which had not undergone any surgical denervation. Responses after pharmacological blockade were also observed in dogs which had undergone selective nerve section. In every case 20-30 minutes elapsed between the last pre-drug occlusion and the first post-drug occlusion. Three responses were always averaged under each experimental condition.

Flow debt and reactive hyperemia flow were obtained by measuring the area under the mean flow curve with a polar planimeter. Percentage repayment of flow debt was obtained by the method of Coffman and Gregg (14). Systolic and diastolic stroke coronary flow was obtained by replaying taped responses at a high recording speed. Systolic flow was obtained by measuring the area under the coronary flow curve during aortic flow and diastolic flow was measured during the remainder of the cardiac cycle.

Venous blood samples were analyzed for dopamine β-hydroxylase (DBH) in 3 dogs by the method of Nagatsu and Undenfriend (15). Samples were taken prior to onset of coronary occlusion and again immediately prior to release. In 2 dogs, the same sampling procedure was used after administration of propranolol.

The student t-test for paired observations was employed to determine significant differences in responses obtained on the same day. Values obtained on different days were compared by analysis of variance.

Results

Cardiovascular changes during one minute occlusion of the left circumflex coronary artery were not significantly different from those already reported (16,17,18,19). Average peak responses of all 26 animals studied include: heart rate (+26 $\pm$ 3 b/min), arterial pressure (-6 $\pm$ 1 mmHg), left atrial pressure (+4.5 $\pm$ 0.6 mmHg), left ventricular dp/dt (-350 $\pm$ 54 mmHg/sec), cardiac output (-320 $\pm$ 55 ml/min), and stroke volume (-7.3 $\pm$ 0.7 ml/b).

Typical left circumflex coronary flow changes associated with one minute occlusion of the artery are demonstrated in Figure 1A. Average replacement/deficit ratios for the 26 dogs studied was 2.68/1. A comparison of systolic and diastolic left circumflex coronary flow was made between control conditions and the peak of the reactive hyperemia. At rest, systolic flow was 21.5% of total flow. At the peak of reactive hyperemia, systolic flow was still 21.5% of total flow. Thus, there was no change in the relationship between systolic and diastolic peak flow following one minute of coronary occlusion (Fig. 2).

Surgical Cardiac Sympathectomy.

Total cardiac sympathectomy was performed in 11 dogs in order to determine a possible role of the cardiac sympathetic

nerves in mediating coronary reactive hyperemia. Sympathectomy did not significantly change resting circumflex coronary flow. However, these dogs did demonstrate much reduced reactive hyperemia when compared with control trials. The averaged control responses for this group was 2.61/1, replacement to deficit, whereas after sympathectomy the response was reduced to 1.67/1 (Figs. 1B, 3). The arterial pressure response was not significantly different after sympathectomy but the heart rate change during occlusion was significantly less after denervation (+30.2 b/min, before; +9.7 b/min, after: $P < .001$).

Three dogs underwent selective left sympathectomy. This procedure did not consistently alter reactive hyperemia and changes which did occur were small. The average replacement to deficit ratios were: 2.38/1 before; 2.30/1 after section (Fig. 3).

The right cardiac sympathetic nerves were selectively sectioned in 5 dogs. In this group, the right ansae subclavia were looped and the incision closed. Prior to recovery from anesthesia both pre- and post right sympathectomy occlusions were performed in the closed-chest dog. Responses were substantially less after nerve section in 4 of the 5 dogs (average of all 5: 1.93/1 before; 1.48/1 after). The change approached but did not meet the criterion for significance at $P < .05$ which was not surprising since the anesthetic apparently had depressed the control response. After recovery from surgery, reactive hyperemia in this group of dogs was significantly

less than the presurgery control (2.25/1 before; 1.38/1 after) (Fig. 3). As in the totally sympathectomized group, the heart rate change during occlusion was significantly less after right sympathectomy (23.0 vs 13.4 b/min, $P < .01$). Subsequent left sympathectomy in this group had no significant effect on the response (1.59/1). Thus, it appears that the right cardiac sympathetic nerves have a direct role in regulation of coronary reactive hyperemia.

Pharmacological Blockade.

The role played by specific cardiac receptors was investigated pharmacologically. First, beta adrenergic blockade was accomplished with propranolol in 22 dogs. Reactive hyperemia was reduced to 1.81/1 (replacement/deficit) which was significantly less than control (2.71, $P < .001$) (Fig. 4, Table 1). As previously reported (17) there were no significant changes in inotropic indices during coronary occlusion which could account for the difference between pre- and post-propranolol responses. Additionally, since the fall in arterial pressure during occlusion was similar in both cases (control -7 mmHg, post-propranolol -9 mmHg), the difference in afterload was not a factor.

Propranolol and cardiac sympathectomy have both been shown to significantly reduce reflex heart rate changes due to coronary occlusion (18). Nevertheless, in this study, trials from 8 dogs were found in which pre- and post-propranolol heart rate responses were similar (less than 6 b/min difference in peak response). These trials were compared to determine

if differences in heart rate responses could account for the difference in reactive hyperemia after propranolol. A significant reduction in reactive hyperemia was observed after propranolol (2.27/1 before, 1.62/1 after, $P < .01$) in these selected occlusions even though there was no significant difference in the average degree of tachycardia. Thus, heart rate differences were not a major factor in the pre- and post-propranolol reactive hyperemia differences.

The relative percent of coronary flow during systole and diastole was determined after propranolol. At rest, systolic flow was 21.2% of total flow. During peak reactive hyperemia, systolic flow contributed 24.8% of total flow. Thus, beta receptor blockade did not alter the phase distribution of circumflex coronary arterial flow.

Vasodilation may potentially be affected by inotropic changes secondary to increased sympathetic nerve activity. Practolol was administered to 9 dogs to selectively block myocardial β_1 receptors and, consequently, abolish any positive inotropic effect. After practolol, the average reactive hyperemia was attenuated from 2.68/1 to 2.36/1 ($P < .05$). Subsequently, propranolol was infused and the reactive hyperemia was further attenuated to 1.66/1 ($P < .001$). On a second experimental day, propranolol was administered first in 6 dogs causing a reduction in reactive hyperemia from 3.07/1 to 2.05/1. Practolol was then given and a further, slight reduction was observed (1.81/1) (Fig. 4). Thus, although a metabolic vasodilatory effect was revealed, the major influence appeared to be via the coronary

β_2 receptors.

Possible involvement of the parasympathetic nervous system was also investigated. Atropine infusion alone in 8 dogs increased heart rate by 82 b/min and mean coronary blood flow by 46% ($P < .001$). The magnitude of reactive hyperemia was reduced by atropine from 2.36/1 to 1.90/1 ($P < .01$). The subsequent addition of practolol did not affect the response, however, propranolol did significantly reduce the response further (Fig. 5). Thus, either directly or indirectly, parasympathetic nerves also appear to influence the magnitude of reactive hyperemia.

After administration of propranolol to those dogs which had previously undergone selective left sympathectomy, the reactive hyperemia was further reduced (Fig. 3). On the other hand, after right sympathectomy, neither practolol nor propranolol plus practolol further reduced the degree of the response (Fig. 3).

Circulating Catecholamines.

The possibility that circulating catecholamines changed during reactive hyperemia was tested by taking blood samples from the left atria of 3 dogs both at rest and during peak reactive hyperemia. Results indicated that circulating dopamine β-hydroxylase were not different between resting and hyperemia samples. It has been demonstrated that dopamine β-hydroxylase is released with catecholamines and it therefore indicates blood levels of catecholamines (15,21). These results indicate that circulating catecholamines are not involved in the hyperemic response following coronary occlusion.

Discussion

Our results indicate that a significant component of the reactive hyperemia observed, due to one minute occlusion of the left circumflex coronary artery, can be accounted for by reflex vasodilation through cardiac beta receptors.

Factors influencing reactive hyperemia are indeed complex. Metabolic factors have been shown to play a major role in control of flow through the coronary vasculature although the exact mechanisms are not at all agreed upon (1,22). Myogenic factors may also play an important role in the regulation of hyperemic flow (9). The nervous system has not previously been studied as a factor in reactive hyperemia, even though it has been shown to produce significant effects on coronary flow (6).

In carefully controlled experiments, direct efferent nerve stimulation has demonstrated neural control of coronary vascular resistance independent of metabolic or myogenic factors (3,4). Activation of the vagus has been shown to produce direct vasodilation and a concomitant increase in coronary sinus PO_2 (4). Our results tend to support this finding in that vagal blockade with atropine did significantly reduce reactive hyperemia. However, our findings do not provide unqualified support since atropine increased resting heart rate and coronary flow dramatically in the conscious dog.

Direct efferent sympathetic nerve stimulation has produced both vasoconstriction and vasodilation. The vasodilation is reported to have two components. First, sympathetic stimulation

has been shown to increase the metabolic needs of the myocardium, thus producing an autoregulatory increase in flow (1). This can be blocked by the selective β_1 blocking agent practolol, which prevents the increased metabolic requirements (23). In our experiments, reactive hyperemia was less after practolol suggesting that metabolic activity of the myocardium was reduced due to the drug. Secondly, sympathetic nerve stimulation has been shown to produce coronary vasodilation independent of a change in metabolic requirements (8,23,24). These data support our findings in that they demonstrate the potential for neurally-mediated coronary vasodilation independent of metabolic changes. Feigl (3), has demonstrated that during direct left sympathetic nerve stimulation, alpha vasoconstriction is the dominant response after total beta blockade. Unfortunately, such a phenomenon could not be investigated in our study due to complicating alterations in arterial pressure after alpha blockade in the conscious dog.

One minute coronary occlusion in the conscious dog has been demonstrated to have dramatic effects on cardiac performance (16,17). This was true both before and after beta receptor blockade or cardiac sympa comy. In each case, inotropic responses to coronary occlusion were not significantly different from each other (17). Similar results were observed in the present experiments. This suggests that coronary occlusion did not produce reflex changes in the inotrop state of the heart, thus, altered metabolic demands could not account for the large component of the reactive hyperemia blocked by propranolol or

cardiac sympathectomy in our experiments. Direct right stellate ganglion stimulation after practolol has been shown to increase coronary blood flow without changing myocardial oxygen consumption (24). Thus, an increase in selective sympathetic neural activity associated with the reactive hyperemia could be a true overpayment leaving a reserve potential for oxygen extraction by the myocardium.

Our results indicate that the efferent pathway for the reflex beta vasodilator effect lies primarily in the right sympathetic nerves. Left cardiac sympathectomy, either before or after right side section, has no apparent affect on reactive hyperemia. Right cardiac sympathectomy or total cardiac sympathectomy produced a large, significant reduction in reactive hyperemia. Such specificity of the pathway is not surprising since the efferent limb of sympathetic mediated heart rate changes in response to coronary occlusion and volume expansion have been found primarily in the right nerves (18,25). This may also explain why direct sympathetic stimulation seldom has provided good support for a reflex sympathetic vasodilator effect. In most previous studies, the left cardiac sympathetic nerves have been stimulated (1,3,8,26). The majority of these studies emphasized an alpha vasoconstrictor influence due to left stellate stimulation. Ross and Mulder (27) showed an interesting separation of effects between stimulation of the right and left cardiac sympathetic nerves before and after propranolol. Before propranolol, end-diastolic vascular resistance in the circumflex coronary artery was decreased by

stimulation of either left (-26%) or right (-19%) cardiac sympathetic nerves. After propranolol, left sympathetic stimulation increased vascular resistance 75%; whereas, right sympathetic stimulation caused only a 9% increase. Such results suggest that alpha effects would be emphasized and beta effects minimized during left cardiac sympathetic nerve stimulation. Further support is found in the study by Nayler and Carson (24) in which right stellate ganglion stimulation after practolol caused coronary vasodilation without a change in myocardial O_2 consumption. In this same study several different β_2 blocking drugs were used and in every case subsequent right stellate stimulation caused a reduction in coronary blood flow.

In our study, curculating catecholamines did not influence the response to coronary occlusion since dopamine beta hydroxide levels did not change. Lack of alterations in circulating catecholamine levels during acute coronary occlusions has been previously observed (28).

In our study, beta blockade did reduce mean coronary flow significantly. It can be presumed that this reduced the metabolic requirements of the heart either through a direct effect on the myocardium or by blocking circulating catecholamine effects. Cardiac sympathectomy, on the other hand, produced no significant fall in coronary flow. In this case both circulating catecholamines and spontaneous release of norepinephrine from postganglionic nerves in the heart may have been significant factors. Previous work has indicated that in conscious

dogs which had undergone both beta blockade and cardiac sympathectomy on separate occasions, beta blockade caused measureable reduction in inotropic indices whereas sympathectomy did not (17). Thus, similar reductions in reactive hyperemia due to coronary occlusion subsequent to either beta blockade or sympathectomy in our study indicates an active vasodilator component under neural control. This phenomenon appears to contribute to the substantial over repayment of coronary blood flow during reactive hyperemia.

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References

1. Berne, RM, De Geest, H, Levy, MN: Influence of the cardiac nerves on coronary resistance. Am J Physiol 208: 763-769, 1965
2. Klocke, FJ, Kaiser, GA, Ross, J, Jr, Braunwald, E: An intrinsic adrenergic vasodilator mechanism in the coronary vascular bed of the dog. Circ Res 16: 376-382, 1965
3. Feigl, EO: Sympathetic contro of coronary circulation. Circ Res 20: 262-271, 1967
4. Feigl, EO: Parasympathetic control of coronary blood flow in dogs. Circ Res 25: 509-519, 1969
5. Vatner, SF, Franklin, D, Van Citters, RL, Braunwald, E: Effects of carotid sinus nerve stimulation on the coronary circulation of the conscious dog. Circ Res 27: 11-21, 1970
6. Rubio, R, Berne, RM: Regulation of coronary blood flow. Prog Cardiovasc Dis 18: 105-122, 1975
7. Feigl, EO: Reflex parasympathetic coronary vasodilation elicited from cardiac receptors in the dog. Circ Res 37: 175-182, 1975
8. Brown, AM: Motor innervation of the coronary arteries of the cat. J Physiol 198: 311-328, 1968
9. Eikens, E, Wilcken, DEL: Myocardial reactive hyperemia and coronary vascular reactivity in the dog. Circ Res 33: 267-274, 1973
10. Bache, RJ, Cobb, FR, Greenfield, JC, Jr: Effects of increased myocardial oxygen consumption on coronary reactive hyperemia in the awake dog. Circ Res 33: 588-596, 1973

TABLE 1 Effects of Selective Sympathectomy on Reactive Hyperemia Repayment After One Minute Circumflex Coronary Occlusions

# Dogs	Control	Left Sympathectomy	Right Sympathectomy	Propranolol	Bilateral Sympathectomy
3‡	2.38 ± .29	2.31 ± .20		1.71 ± .34	
5	2.25 ± .29		1.38 ± .27***	1.25 ± .28**	1.47 ± .31*
11	2.62 ± .23			1.64 ± .17***	1.67 ± .24***

Surgery was performed in the sequence indicated for each group of dogs. Each column represents data collected on separate experimental days except in the case of propranolol in the first two groups which was obtained on the same day as either left or right sympathectomy data. In each case significance represents statistical comparison with the control values *$P < .05$, **$P < .01$, *** $P < .001$. ‡ Statistical analysis was not attempted on these 3 dogs nor was bilateral sympathectomy carried out on this group.

11. Bach, RJ, Cobb, RF, Greenfield, JC. Limitation of the coronary vasoconstriction in the dog. Circ Res 37: 88-95, 1974

12. Debley, VG: Miniature hydraulic occluder for zero blood flow determination. J Appl Physiol 31: 138-139, 1971

13. Mizeres, NJ: Origin and course of the cardioaccelerator fibers in the dog. Anat Rec 132: 261-279, 1958

14. Coffman, JD, Gregg, DE: Reactive hyperemia characteristics of the myocardium. Am J Physiol 199: 1143-1149, 1960

15. Nagatsu, T, Udenfriend, S: Photometric assay of dopamine-β-hydroxylase activity in human blood. Clin Chem 18: 980-983, 1972

16. Bishop, VS, Kaspar, RL, Barnes, GE, Kardon, MB: Lef ventricular function during acute regional myocardial ischemia in the conscious dog. J Appl Physiol 37: 785-792, 1974

17. Kaspar, RL, Barnes, GE, Peterson, DF, Bishop, VS: Specificity of autonomic influences on cardiac responses during myocardial ischemia. J Appl Physiol 38: 485-490, 1975

18. Peterson, DF, Kaspar, RL, Bishop, VS: Reflex tachycardia due to temporary coronary occlusion in the conscious dog. Circ Res 32: 652-659, 1973

19. Peterson, DF, Bishop, VS: Reflex blood pressure control during acute myocardial ischemia in the conscious dog. Circ Res 34: 226-232, 1974

20. Reigl, EO: Control of myocardial oxygen tension by sympathetic coronary vasoconstriction in the dog. Circ Res 37: 88-95, 1975

21. Echanberg, SM, Stone, RA, Kirshner, N, Gunnells, JC, Robinson, RR: Plasma dopamine-β-hydroxylase: A possible aid in the study and evaluation of hypertension. Science 183: 523-525, 1974

22. Olsson, RA: Myocardial reactive hyperemia. Circ Res 37: 263-269, 1975

23. Hamilton, FN, Feigl, EO: Coronary vascular sympathetic beta-receptor innervation. Am J Physiol 230: 1569-1576, 1976

24. Nayler, WG, Carson, V: Effect of stellate ganglion stimulation on myocardial blood flow, oxygen consumption, and cardiac effeciency during beta-adrenoreceptor blockade. Cardiovas Res 7: 22-29, 1973

25. Bishop, VS, Peterson, DF: Pathways regulating cardiovascular changes during volume loading in awake dogs. Am J Physiol 231: 854-859, 1976

26. Granata L, Olsson, RA, Huvos, A, Gregg, DE: Coronary inflow and oxygen usage following cardiac sympathetic nerve stimulation in unanesthetized dog. Circ Res 16: 114-120, 1965

27. Ross, G, Mulder, DG: Effects of right and left cardiosympathetic nerve stimulation on blood flow in the major coronary arteries of the anesthetized dog. Cardiovasc Res 3: 22-29, 1969

28. Toltzis, RJ, Satinsky, VP, Reed, V, Angelakos, ET, Scott, JC: Effects of coronary artery occlusion with a swan-ganz catheter on plasma norepinephrine. Fed Proc 33: 413, 1974

Legends

FIGURE 1 Left circumflex coronary flow associated with one minute occlusion. The top trace is pulsatile flow, the bottom trace is mean flow. Resting mean flow is indicated by the dashed line. Part A is the response in the control state; part B is after recovery from total cardiac sympathectomy.

FIGURE 2 Comparison of systolic and diastolic components of coronary flow (CF) at rest and during peak reactive hyperemia. The top trace is pulsatile aortic flow.

FIGURE 3 Effects of cardiac sympathectomy on over repayment during coronary reactive hyperemia. Values represent percentage repayment above unity. C, control; T.S., total sympathectomy; L.S., left sympathectomy; R.S., right sympathectomy; Pp, propranolol blocked; Pt, practolol blocked; .001 p value compared to preceeding observation.

FIGURE 4 Effects of blocking agents on reactive hyperemia. Statistical comparisons are made with the preceeding value. C, control; Pp, after propranolol; Pt, after practolol.

FIGURE 5 Effects of blocking agents on reactive hyperemia. C, control; At, atropine; Pt, practolol; Pp, propranolol; $**P < .01$ compared with preceeding value.

Figure 1

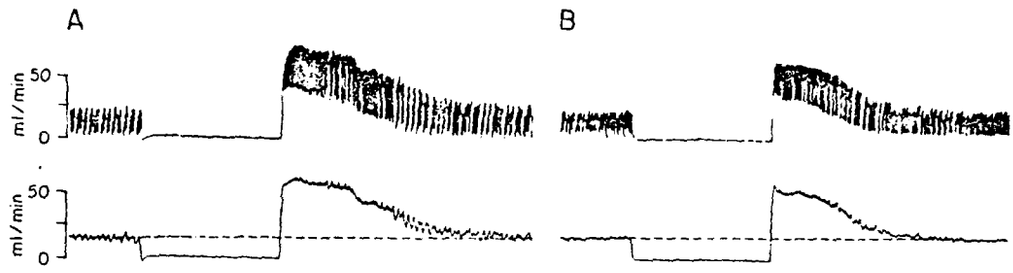


Figure 2

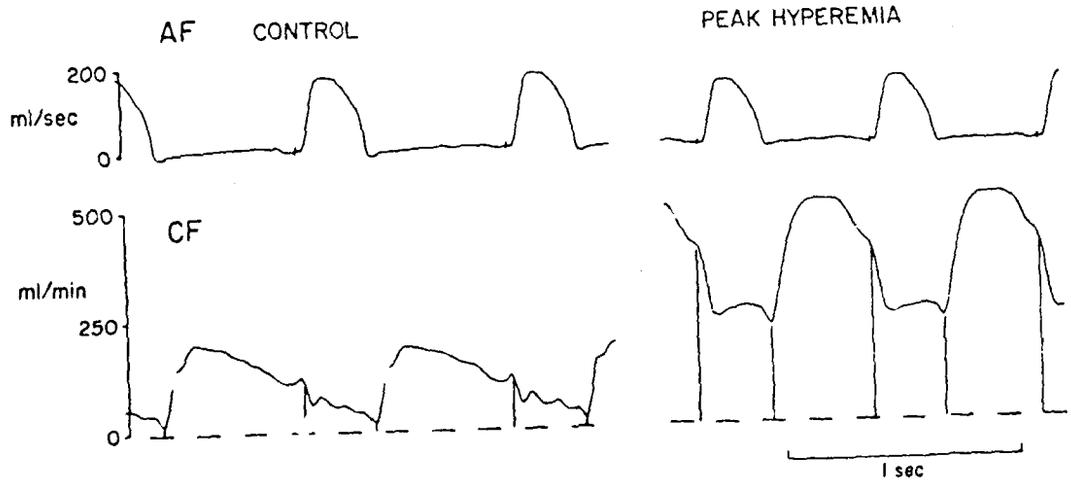


Figure 3

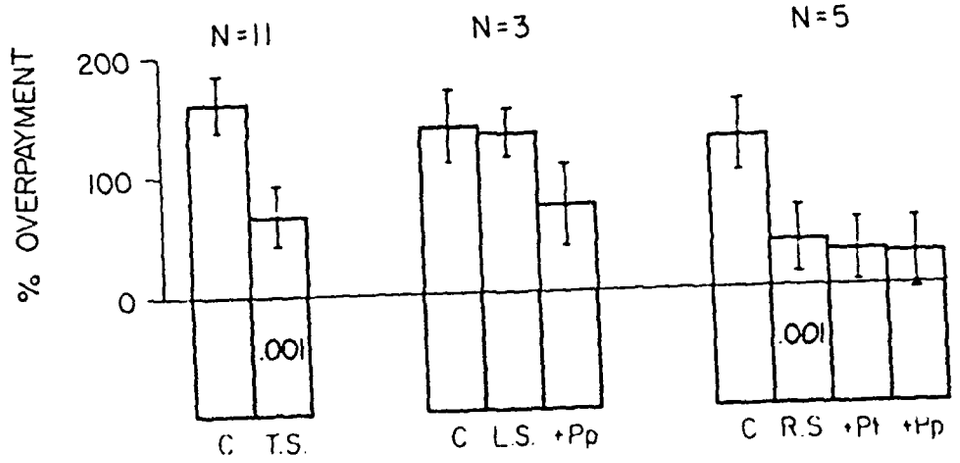


Figure 4

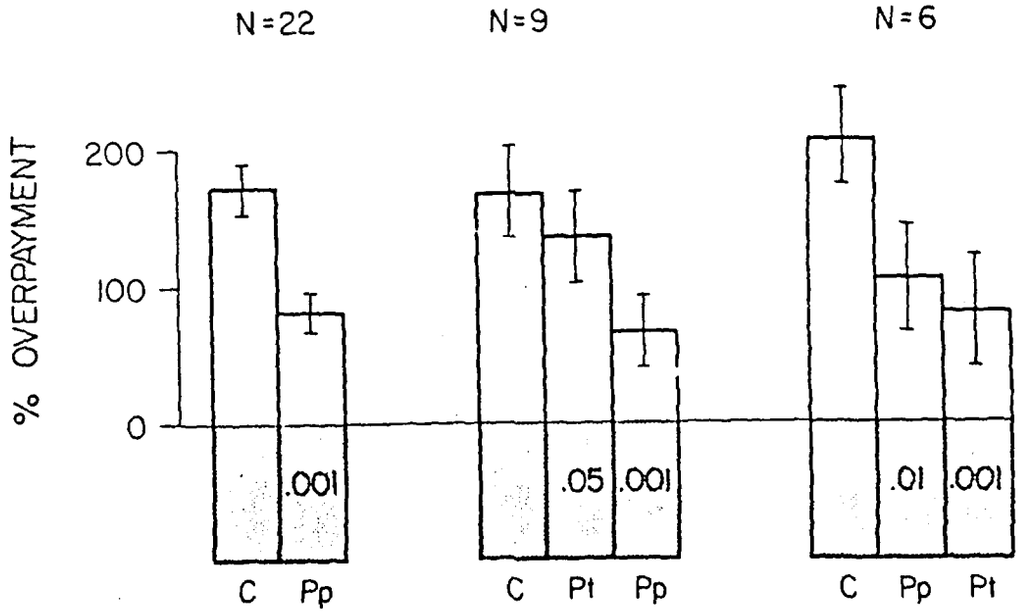
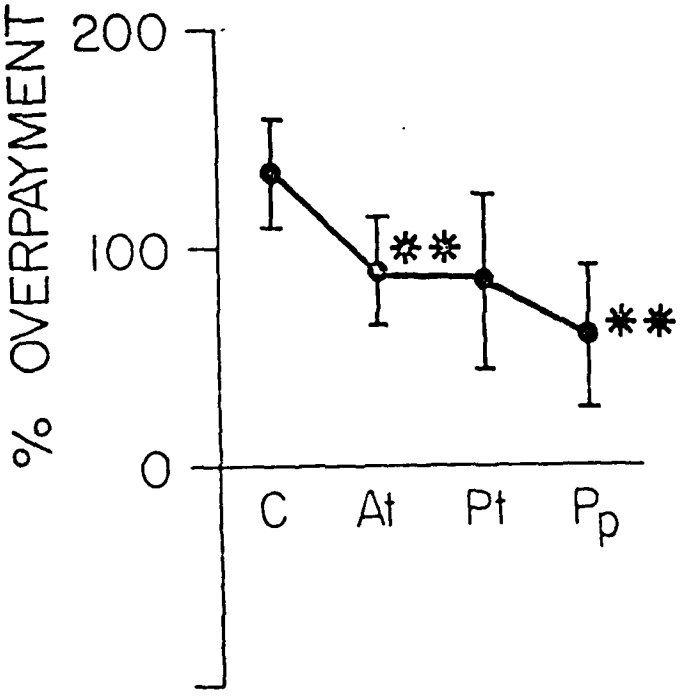


Figure 5



Rabbit Cardiovasculer Responses During Vasoactive Drug Infusion at Fixed Carotid Pressure

By

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Abbreviated Title: Baroreflex alteration to vasoactive drugs

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ABSTRACT

In anesthelized rabbits, peak reflex brodycardia (Δ HR) and depression of mean arterial blood pressure (Δ MAP) were measured during maximal central stimulation of the left aortic nerve (LANS). Responses were quantified; (i) before and during steady state changes (± 15 mm Hg) in the isolated carotid intrasinus pressure (ISP), and (ii) with ISP excluded from the circulation and maintained at a normotensive level (EP = ISP = MAP) the MAP was changed ± 20 mm Hg by the infusion of either nitroglycerin (NG), lysine vasopression (LDH) or phenylephrine (PE). Results indicated that within ± 15 mm Hg of EP, the change in MAP per mm Hg change in ISP was 3, while Δ MAP due to LANS changed nearly double per mm Hg change in ISP. Following vagotomy a small increase in MAP was seen, however, cardiovascular responses to changes in ISP or LANS were unaltered. During drug infusion with the carotid sinuses excluded from the circulation and ISP = EP, peak Δ HR and Δ MAP to LANS were independent of the direction or magnitude of the drug induced change in MAP. When carotid baroreceptors were allowed to detect the increase in MAP, the peak Δ HR and Δ MAP responses to LANS were significantly reduced. These results suggest a high degree of sensitivity of the carotid sinus baroreceptors around the animal's normotensive region and that activity from these baroreceptors can modify reflex vascular tension even in the absence of significant change in heart rate or arterial pressure.

Index terms: baroreflex change, vasoactive drugs, aortic nerve stimulation

INTRODUCTION

Many studies have characterized the heart and vascular responses to a wide range of controlled pressures in the isolated carotid or aortic sinus, and defined the response relationship as the sensitivity of the respective baroreflex system (1, 5, 6, 8, 12, 13, 21, 22). Recent studies have characterized low pressure cardiopulmonary mechano-receptors (2, 11, 14, 15, 17, 18, 19, 20, 25, 26) which, when activated, may alter arterial baroreceptor sensitivity. Vatner, et al. (27) found that the reflex heart rate response to a pressure challenge was reduced during venous volume loading and suggested that the set point or gain of the arterial baroreflexes was altered as atrial pressure was raised. Indeed more recent studies (14), have indicated that volume infusion may actually have a direct effect on the arterial baroreflex through subtle cardiovascular alterations. This was apparent when infusion attenuated reflex responses to left aortic nerve stimulation (LANS) were abolished following carotid sinus baroreceptor denervation but not vagotomy. Additionally, since heart rate (HR) and mean arterial pressures (MAP) were not altered by infusion, the usual assumption that an absence of a change in HR or MAP is an indication of a constant carotid baroreceptor input may not be valid. For this reason, it was postulated that the carotid sinus baroreceptors are able to detect subtle changes in pressure or volume which may lead to altered reflex sympathetic efferent activity without significantly altered arterial pressure. This may lead to changes in carotid sinus buffering capacity at apparently unchanged or only slightly changed blood pressures.

The purpose of this study was to examine this postulate by determining whether or not reflex HR and MAP responses to LANS are altered as a result of a change in the carotid sinus buffering activity. Responses were quantified first during controlled carotid sinus pressure disturbances within a physiological range of normotensive arterial pressures. Then, vasoactive drugs were used to change arterial pressure during conditions in which the carotid sinuses pressure was held constant and then subsequently allowed to compensate for the drug induced change in systemic blood pressure.

METHODS

Twenty-two rabbits of either sex (2.7 ± 0.2 kg) were anesthetized with sodium pentobarbital via an ear vein (Diabutal, Diamond Laboratories, Inc., 30 mg/kg IV). A light level of surgical anesthesia was maintained by supplemental administration through a cannulated femoral vein. The descending aorta (via femoral artery) and the right atrium (via the jugular vein) were also cannulated and connected to Statham P23Db and P23BB strain gauges for recording of arterial and right atrial blood pressures, respectively. Heart rate was recorded via sternal needle electrodes connected to a Beckman 984B cardiotachometer coupler. Blood pressure and heart rate were initially recorded on a Beckman R411 oscillograph with parallel output signals to a DEC PDP 8/E Digital computer. The trachea was cannulated via a tracheostomy and each animal breathed spontaneously throughout the experiment. Frequent small arterial blood samples were analyzed for pO_2, pCO_2 and pH on a Beckman IL 113 blood gas

analyzer to assure maintenance of physiological levels for blood gases and pH. Through a midventral incision, the left and right aortic and vagus nerves were located in the cervical region, carefully isolated from surrounding tissue for about 1 cm, and looped with a loose thread for identification prior to sectioning.

Carotid Sinus Isolation:

A modified Moisseieff technique was used to permit reversible isolation of a carotid sinuses from the systemic circulation (21, 22). Both internal carotids were tied at approximately 0.5 cm from their points of origin. The external carotid artery was carefully isolated but remained patent. All other small vessels were ligated. Following heparinization (4 mg/kg IV, 150 units/mg, Hynson, Westcott, Dunning, Inc., Baltimore, Maryland 21201) the carotid arteries were cannulated below the sinus with an extracorporeal loop. This permitted self perfusion of the sinus by the carotids or reversible pressure regulation of the sinuses via an elevated pressure bottle after occlusion of the external carotid arteries. Under all conditions intrasinus pressure (ISP) was monitored by a pressure transducer in the extracorporeal system near the sinus.

In most animals, the sensitivity of the carotid sinus reflex in the control of MAP was evaluated at $\pm$ 15 mm Hg of the equilibrium pressure. This was done by observing the reflex changes in steady state MAP as a result of small step changes in the isolated carotid sinus pressure. Thus, when holding ISP constant, it was assumed that the sensitivity of carotid

sinus was not altered during that portion of the experimental protocol.

Nerve Stimulation:

Both the right and left aortic nerves were cut and the central end of the left placed on bipolar hook electrodes, platinum-iridium, for electrical stimulation (9, 16). When not being stimulated, the nerve was placed on saline soaked cotton to prevent drying. Responses to left aortic nerve stimulation (LANS) were observed both with the carotid sinuses in the circulation and with them isolated and their pressure maintained constant at the predetermined equilibrium pressure. Responses to LANS were obtained under the above conditions both before and after vagotomy and after drug induced alterations in blood pressure (see below).

Electrical stimulation was generated via a Grass S88 stimulator activated by the Schmidt trigger of the computer which was synchronized with the R-wave of the ECG (10,23). Regulation of the stimulus timing and stimulus parameters, as well as continuous calculation of the length of each R-R interval and beat-to-beat mean arterial and mean intrasinus pressure, were accomplished using the computer and special computer program systems. Description of the electrical stimulation parameters used in an experimental trial in this study has been previously published (23). Briefly, stimulation was carried out over 120 cardiac cycles, each burst of electrical activity was made up of ten square wave impulses (ten volts) inserted 10 msec after the recorded R-wave of the ECG. The impulse duration was 0.6 msec, the stimulus frequency was 80 Hz and burst duration was 113 msec.

Drug Infusions:

In ten aortic denervated animals, after obtaining control data, reflex cardiovascular responses were studied following systemic infusion of a pressor or depressor drug to obtain a small (approx. 20 mm Hg) rise or fall in steady state MAP. Drug infusion was initiated (Harvard syringe pump: Harvard Apparatus Co., Inc., Millis, Mass) with the carotid sinuses isolated from the system and held at a pressure equal (EP) to the control mean arterial pressure of that animal. Following the steady state alteration in systemic arterial pressure induced by the vasoactive drug, the LAN was stimulated. Following recovery and while drug infusion continued at a steady rate, the animal was allowed to perfuse his own carotid sinuses (carotid sinuses were included in the circulation) and LAN stimulation repeated. Systemic arterial pressure was increased by IV infusion of phenylephrine hydrochloride (Winthrop Laboratories, Division of Sterling Drugs, Inc., New York, NY) or Lysine vasopressin (Sandoz Pharmaceutical, Hanover, NJ). Decreases in systemic arterial pressure were produced by IV infusion of nitroglycerin (Eli Lilly & Co., Indianapolis, Indiana). Dosages were adjusted for desired effect (volumes, times and amounts are tabulated in Table I). All values are reported as a mean or mean difference $\pm$ the standard error of the mean (SEM). Statistical evaluation was made by use of the appropriate student t-test for paired or unpaired comparisons. P values ($p < 0.05$) were considered significant.

RESULTS

The average HP and MAP values together with the reactive reflex

responses to left aortic nerve stimulation (LANS) are summarized in Table 2. With vagi intact, excluding the carotid sinus from the circulation at equilibrium pressure increased the magnitude of the reflex bradycardia due to LANS from -41 to -50 beats/min. Removal of the vagi decreased the magnitude of the reflex bradycardia when the carotid sinuses were included in the circulation, but had no significant effect when the sinuses were excluded. Thus, regardless of whether or not the vagi are intact, the reflex HR response to LANS is enhanced when the carotid sinuses are prevented from sensing the reflex induced changes in MAP. Excluding the carotid sinuses from the circulation had a small, significant effect on the reflex fall in MAP due to LANS, extending the fall from -48.2±2.6 mmHg to -54.7±2.2 mmHg ($p < 0.05$). With vagi cut, the small extension of the reflex fall in MAP was not significant.

Effect of altered carotid sinus pressure on arterial pressure response to left aortic nerve stimulation (LANS):

In order to evaluate how changes in carotid sinus pressure may alter the response to aortic nerve stimulation, peak change in arterial pressure and heart rate responses to LANS were observed as pressure in the isolated carotid sinus was altered in eight rabbits (Fig. 1 and 2). Pressure within the sinus was altered in small steps between ± 15 mmHg of the equilibrium pressure for each animal. Within this range there was a near linear relationship between carotid sinus pressure and the peak fall in MAP to LANS. This relationship was not altered in seven of the same animals following vagotomy. Thus, nonpulsatile steady state change in carotid intrasinus pressure resulted in reflex modification of the arterial pressure responses to aortic nerve

stimulation. The extent of bradycardia during LANS, however, was unaltered during ± 15 mmHg changes in ISP around the equilibrium pressure. Furthermore, while vagotomy reduced the peak bradycardia to LANS, no relationship of peak bradycardia to ISP level was observed following vagal denervation.

The effects of pressor and depressor agents on the MAP reflex response to LANS stimulation:

Lysine Vasopressin (ADH): With the carotid sinuses excluded (Ex) from the circulation, the intravenous infusion of ADH (a non-specific pressor agent) was adjusted to increase the MAP by about 20 mmHg, (av. 95.7±3.0 to 112.1± 3.6 mmHg) (Fig. 3A). Subsequent reflex fall in MAP to stimulation of the aortic nerve (LANS) was not significantly changed (preinfusion -56.0±3.4 and during infusion -51.0±4.1 mmHg, respectively). With the infusion maintained, the carotid sinuses were included (I) back into the systemic circulation. Mean arterial pressure then decreased to [illegible] mmHg; a value not significantly different from the preinfusion value [illegible] mmHg). However, during drug infusion in the (I) condition, the reflex MAP response to LANS was significantly less (-31.9 ± 3.2 mmHg) compared to the response prior to drug infusion (-51.3 ± 2.7 mmHg).

Phenylephrine: A constant intravenous infusion of phenylephrine (PE), with the carotid sinuses excluded from the circulation, increased the MAP from 96 ± 1.9 to 118 ± 2.6 mmHg (Fig. 3C). Under these conditions, the MAP response (-38.4 ± 6.4 mmHg) to LANS was significantly ($p < 0.05$) reduced from that observed with the carotid sinuses excluded from the circulation without PE infusion (-57.3 ± 3.8 mmHg). With the

carotid sinuses included in the circulation and the infusion of PE maintained constant, MAP was reduced to 108.3 ± 3.2 mmHg, a level significantly ($p < 0.05$) greater than that observed with the carotid sinuses included in the circulation prior to the infusion of PE (96.7 ± 1.6 mmHg). The reflex response to LANS was -30.4 ± 4.3 mmHg which was also significantly less than that observed during LANS prior to PE infusion with carotid sinuses included in the circulation.

Nitroglycerin: Nitroglycerin (NTG) infusion (Fig. 3B) with the carotid sinuses excluded from the circulation, reduced MAP from 96.3 ± 1.5 to 82.9 ± 2.9 mmHg ($P < 0.05$). Including the carotid sinuses into the circulation resulted in a significant ($P < 0.05$) increase in MAP to 92.1 ± 3.0 mmHg. However this value was still significantly ($P < 0.05$ below the preinfusion (N) condition average of 99.4 ± 1.8 mmHg. Yet, during NTG infusion the MAP reflex responses to LANS with or without the carotid sinuses in the circulation were not significantly different, -51.3 ± 2.1 and -50.7 ± 3.9 mmHg respectively.

In all animals MAP was significantly increased a small amount following vagotomy. However, the relative MAP responses to isolated carotid sinus pressure, vasoactive drug infusion and LANS were not altered when compared to corresponding prevagotomy responses.

The effects of pressor agents on the MP reflex responses to LANS stimulation:

Intravenous infusions of ADH necessary to elevate the arterial pressure to the above desired level, resulted in a slight decrease in heart rate (Fig. 3D) even though the carotid sinuses were excluded from the circulation. Including

the carotid sinuses in the circulation produced a significant ($p < 0.05$) reflex decrease in HR from 272 ± 3 to 249 ± 36. With carotid sinuses excluded from the circulation, the reflex bradycardia to LANS was unaltered. However, once the carotid sinuses were allowed to sense the elevated pressure, the reflex bradycardia to LANS stimulation was reduced from -46 ± 1 to -35 ± 4 ($p < 0.05$). Vagotomy reduced the reflex bradycardia to LANS. However, qualitatively the changes observed after vagotomy were similar to that with the vagi intact.

The effects of PE on the resting HR (Fig. 3F) were similar to those observed during ADH infusion. With carotid sinuses excluded PE infusion reduced the HR from 272 ± 6 to 260 ± 6 b/min. When the carotid sinuses were allowed to sense the pressure changes due to PE infusion, the HR was significantly ($p < 0.05$) reduced when compared to the same experimental conditions prior to PE infusion (from 274 ± 6 to 246 ± 5). Reflex bradycardia to LANS was significantly ($p < 0.05$) reduced, from the pre-infusion (1) value, during PE infusion only when the carotid sinuses were included in the circulation (-64 ± 7 to -43 ± 6 b/m respectively). These results contrast to the MAP responses to LANS, which were reduced during PE infusion both before and following inclusion of the carotid sinuses in the circulation. Again, vagotomy did not alter the relative relationships of the responses.

Nitroglycerin: Nitroglycerin had little effect on the resting HR or the reflex HR response to LANS stimulation whether or not the carotid sinuses were included in the circulation (Fig. 3E). As noted previously, vagotomy reduced the reflex HR response to LANS. However, vagotomy did not alter the quantitative aspects of the reflex HR responses when comparisons are made before, during or after isolation of the carotid sinuses.

DISCUSSION

Results of this study demonstrate that the reflex responses to left aortic nerve stimulation (LANS) are dependent upon the steady state carotid sinus pressure. At intrasinus pressures (ISP) 1 to 15 mm Hg above the equilibrium pressure (EP), were ISP and MAP are equal, the reflex fall in mean arterial pressure (MAP) during LANS was diminished. At ISP levels 1 to 15 mm Hg below EP the responses to LANS were enhanced when compared to responses during control conditions. Reduction of reflex bradycardia to LANS, as seen during pressor drug infusion, required exposure of the carotid sinus baroreceptor to pressures in excess of 15 mm Hg higher than EP. These results suggest that the ISP threshold pressure for vascular change in the rabbit is in the normotensive region while that for heart rate change is higher. Such findings are consistent with those previously reported for the dog (1, 4, 6, 21) and the rabbit (Chen et al, unpublished observations).

When bilateral carotid sinus pressure was held constant and excluded from the circulation, the cardiovascular responses to LANS were independent of the MAP increase produced by constant infusion of lysine vasopressin (ADH). When the carotid sinuses were allowed to sense the ADH produced pressure change, systemic arterial pressure returned almost to the control level. Now reflex fall in MAP due to LANS was significantly reduced when compared to the preinfusion control responses. These results suggest that the reflex adjustments by the carotid sinus which decrease arterial pressure during ADH infusion resulted in a net reduction in vasomotor

activity; even though the blood pressure was essentially the same as the preinfusion MAP.

During phenylephrine (PE) infusion with the carotid sinuses isolated at equilibrium pressure, the average MAP rose significantly, however, reflex fall in MAP to LANS was significantly reduced. These results are in sharp contrast to those during ADH-infusion. Re-exposing the carotid sinuses to the systemic pressure, during PE infusion, resulted in an additional attenuation of the MAP response to LANS. These results suggest that, unlike ADH, a nonspecific vasoconstrictor, phenylephrine produces an increase in arterial pressure by competing with norepinephrine for alpha receptors. Consequently, when sympathetic activity is reflexly withdrawn by LANS, the available phenylephrine occupies more receptor sites and the reflex fall in arterial pressure is limited. This limitation is also observed when the carotid sinuses are permitted to attempt a reflex decrease in MAP during PE infusion. Thus, in the presence of substantially reduced sympathetic output to resistance vessels, further withdrawal due to LANS appears to be even less.

Attenuation of the reflex HR response during ADH and PE infusions were also observed when the carotid sinus were included into the circulation. Unlike the MAP responses to LANS observed with PE infusion, no attenuation was observed in the HR response to LANS when the carotid sinuses were excluded from the circulation, lending additional support for the alpha agonist effect of PE. In addition, although vagotomy reduced the magnitude of the reflex HR response, it did not qualitatively alter the

degree of attenuation of the HR response to the pressor agents when the carotid sinuses were included in the circulation. Thus, the attenuation of the HR response to LANS occurred, apparently as a secondary result of carotid sinus adjustments to the pressure increases which reduced sympathetic efferent activity (7). The parasympathetic component for reflex heart rate change was apparently unaltered. This agrees with a previous study in which the attenuation of the HR response to LANS during volume loading was also found to be dependent upon the sympathetic efferent activity (24).

Intravenous infusion of nitroglycerin (NTG) resulted in a fall in MAP when the carotid sinuses were excluded from the circulation both before and following vagotomy. The reflex fall in MAP to LANS was not significantly altered during infusion when the responses were compared to pre-infusion values. Exposure of the carotid sinuses to NTG induced hypotension caused a significant rise in arterial pressure. However, MAP remained somewhat reduced when compared to pre-infusion control values. In addition, the reflex fall in arterial pressure due to LANS during NTG infusion, while the carotid sinuses sensed systemic pressure, was not significantly different from the control responses prior to drug infusion, when the carotid sinuses were included in the circulation. These results would not have been predicted from the earlier carotid sinus isolation data (Fig. 1A,B). That is, with carotid sinus pressure controlled, it was demonstrated that pressure responses to LANS were enhanced during low carotid sinus pressure and diminished at high carotid sinus pressures.

This relationship was linear within the near normotensive range of pressures tested. From this relationship, one would expect that when pressure was lowered by infusion of a nonspecific vasodilator, such as nitroglycerine, there would be an enhancement of the vascular response to LANS. This was not observed. This discrepancy is further surprising in view of previous results from our laboratory in which the reflex HR and MAP responses to LANS were significantly increased after total arterial baroreceptor denervation (24). In this earlier work, total arterial baroreceptor denervation significantly increased the resting MAP indicating a higher initial sympathetic efferent activity. Important, however, is that under conditions of total arterial baroreceptor denervation, the carotid sinuses cannot reflexly adjust to the falling MAP during LANS. In this study, with the carotid sinuses isolated, with ISP held at EP, infusion of NTG resulted in a relatively small decrease in MAP. It is unlikely then, that this drop in MAP when detected by the intact carotid baroreceptors resulted in an input to the CNS equivalent to total carotid sinus denervation. Furthermore, from the first part of this study it can be assumed that the carotid baroreflex mechanisms are operating within normotensive sensitivity levels during NTG infusion. Therefore, a possible explanation of the apparent contradiction of the responses to LANS during NTG infusion to those predicted, may be differences in compensatory mechanisms working when pressure is lowered by vasodilation versus when it is raised by vasoconstriction. That is, in the case of carotid sinus adaptation to a drug-induced rise in pressure, the net effect is a reduction in peripheral sympathetic tone. Subsequently, further withdrawal of peripheral sympathetic tone

through aortic nerve stimulation would be less effective as indicated by our results. This response would result from a combination of the initial lower resting sympathetic tone and the buffering effect of the carotid sinus as pressure falls. However, when pressure is lowered by a vasodilator and partially corrected by the carotid sinuses peripheral sympathetic activity is elevated. LANS causes withdrawal of sympathetic output but as pressure falls, the decrease in carotid sinus activity opposes the sympathetic withdrawal and tends to prevent an enhancement of the effect predicted at a reduced isolated carotid sinus pressure.

Heart rate responses to LANS were not altered due to NTG when responses during infusion are compared to appropriate control responses prior to infusion, either before or after vagotomy. These results were essentially expected when one considers the results of the heart rate and bradycardia responses to LANS during the small changes in isolated carotid intrasinus pressure as discussed earlier.

Finally, the use of vasoactive pharmacological agents may involve direct and indirect effects which could result in more complex interactions then discussed above. For example, possible factors in our results during NTG infusion could include a direct effect of the drug on the smooth muscle within the carotid sinus region resulting in a change in the activity or configuration of the near by baroreceptor as well as an altered reflex mechanoreceptor influence in the cardiopulmonary region during NTG dilation of vessels in these areas (3, 19).

References

1. ALLISON, IL, K. SAGAWA, M. KUMADA: An open-loop analysis of the aortic arch barostatic reflex. Am J Physiol 217 (6): 1576-1584, 1969.

2. BISHOP, VS, F. LOMBARDI, A. MALLIANI, M. PAGANI, G. RECORDATI: Reflex sympathetic tachycardia during intravenous infusions in chronic spinal cats. Am J Physiol 230 (1): 25-29, 1976.

3. COHEN, MV, ES. KIRK: Differential response of large and small coronary arteries to nitroglycerin and angiotensin. Circ Res 33: 445-453, 1973.

4. COWLEY, AW Jr, E. MONOS, AC. GUYTON: Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circ Res 34: 505-514, 1974.

5. EDIS, AJ: Aortic baroreflex function in the dog. Am J Physiol 221 (6): 1352-1357, 1971.

6. HAINSWORTH, R, JR. LEDSOME, F. CARSWELL: Reflex responses from aortic baroreceptors. Am J Physiol 218 (2): 423-429, 1970.

7. HEYNDRICKX, GR, DH. BOETTCHER, SF. VATNER: Effects of angiotensin, vasopressin, and methoxamine on cardiac function and blood flow distribution in conscious dogs. Am J Physiol 231 (3): 1579-1587, 1976.

8. HOCKMAN, CH, J. TALESNIK: Central nervous system modulation of baroreceptor input. Am J Physiol 221: 515-519, 1971.

9. KARDON, MB, DF. PETERSON, VS. BISHOP: Reflex bradycardia due to aortic nerve stimulation in the rabbit. Am J Physiol 225 (1): 7-11, 1973.

10. KARDON, MB, DF. PETERSON, VS. BISHOP: Reflex heart rate control via specific aortic nerve afferents in the rabbit. Circ Res 37: 41-47, 1975.

11. KOIKE, H, AL. MARK, DD. HEISTAD, PG. SCHMIDT: Influence of cardiopulmonary vagal afferent activity on carotid chemoreceptor and baroreceptor reflexes in the dog. Circ Res 37: 422-429, 1975.

12. KUMADA, M, K. SAGAWA: Aortic nerve activity during blood volume changes. Am J Physiol 218 (4): 961-965, 1970.

13. LEVY, MN, ML. NG, H. ZIESKE: Cardiac and respiratory effects of aortic arch baroreceptor stimulation. Cir Res 19: 930-939, 1966.

14. MANCIA, G, DE. DONALD, JT. SHEPHERD: Inhibition of adrenergic outflow to peripheral blood vessels by vagal afferents from the cardiopulmonary region in the dog. Cir Res 33: 713-721, 1973.

15. MANCIA, G, JT. SHEPHERD, DE. DONALD: Interplay among carotid sinus, cardiopulmonary, and carotid body reflexes in dogs. Am J Physiol 230 (1): 19-24, 1976.

16. NEIL, E, CRM. REDWOOD, A. SCHWEITZER: Effects of electrical stimulation of the aortic nerve on blood pressure and respiration in cats and rabbits under chloralose and nembutal anesthesia. J Physiol (Lond) 109: 392-401, 1949.

17. OTT, NT, JT. SHEPHERD: Vasodepressor reflex from lung inflation in the rabbit. Am J Physiol 221 (3): 889-895, 1971.

18. PELLETIER, CL, AJ. EDIS, JT. SHEPHERD: Circulatory reflex from vagal afferents in response to hemorrhage in the dog. Circ Res 29: 626-634, 1971.

19. PELLETIER, CL, JT. SHEPHERD: Circulatory reflexes from mechanoreceptors in the cardio-aortic area. Circ Res 33 (2): 131-136, 1973.

20. PILLSBURY, HRC III, M. GUAZZI, ED. FPEIS: Vagal afferent depressor nerves in the rabbit. Am J Physiol 217 (3): 766-770, 1969.

21. SCHMIDT, RM, M. KUMADA, K. SAGAWA: Cardiac output and total peripheral resistance in carotid sinus reflex. Am J Physiol 221(2): 480-487, 1971.

22. SCHMIDT, RM, M. KUMADA, K. SAGAWA: Cardiovascular responses to various pulsatile pressures in the carotid sinus. Am J Physiol 223 (1): 1-7, 1972.

23. STINNETT, HO, DF. PETERSON, VS. BISHOP: Cardiovascular responses to electrocardiogram-coupled stimulation of rabbit aortic nerve. Am J Physiol 230 (5): 1374-1378, 1976.

24. STINNETT, HO, VS. BISHOP, DF. PETERSON: Reduction of baroreflex cardiovascular responses due to venous infusion in the rabbit. Circ Res 39 (6): 766-772, 1976.

25. THOREN, PN, G. MANCIA, JT. SHEPHERD: Vasomotor inhibition in rabbits by vagal nonmedullated fibers from cardiopulmonary area. Am J Physiol 229 (5): 1410-1413, 1975.

26. UCHIDA, Y: Afferent sympathetic nerve fibers with mechanoreceptors in the right heart. Am J Physiol 223 (1): 203-207, 1972.

27. VATNER, SF, DH. BOETTCHER, GR. HEYNDRICKX, RJ. McRITCHIE: reduced baroreflex sensitivity with volume loading in conscious dogs. Circ Res 37: 236-242, 1975.

Table 1

Concentration and average (± SEM) values for the total volume (TV) and length of time (T) for drug administration in ten aortic denervated rabbits with vascularly isolated carotid sinuses during open and closed loop conditions.

	Wt. (Kg)	Drug	Concentration	TV (ml)	T (m)
Vagi Intact	2.52 ± 0.10	ADH	4.5 mU/ml	7.2 ± 1.4	7.1 ± 0.8
		NTG	11.2 ug/ml	6.2 ± 1.5	6.5 ± 0.7
		PE	35.0 ug/ml	3.6 ± 0.3	5.3 ± 0.3
Vagi Cut		ADH	same	8.7 ± 1.3	7.2 ± 1.5
		NTG	same	7.9 ± 1.9	7.7 ± 0.9
		PE	same	5.4 ± 1.3	6.8 ± 0.9

NTG = nitroglycerin; ADH = lysine vasopressin; PE = phenylephrine hydrochloride.

Table 2

Rabbit heart rate (HR) and mean arterial pressure (MAP) responses (± SEM) and peak changes (Δ) to LANS during circulatory inclusion (I) and exclusion (Ex) of the carotid sinus.

Parameter	-ANs +Vagi				-ANs -Vagi			
	I	Δ	Ex-EP	Δ	I	Δ	Ex-EP	Δ
HR (b/m)	258 (9)	41.0 (5.8)	260 (8)	49.8* (6.0)	263 (7)	31.5* (4.6)	267 (8)	45.6* (4.5)
MAP (mmHg)	108.5 (3.2)	48.2 (2.6)	101.3* (2.7)	54.7* (2.2)	113.5* (3.3)	51.0 (2.7)	105.6* (2.4)	55.5 (2.1)
N=	15		15		14		14	

-ANs = both aortic nerves sectioned; (Ex-EP) isolated carotid sinus pressure held equal to MAP; LANS = stimulation of central end of left aortic nerve; N = number of animals; -Vagi = vagi intact; * = significantly different from the prior closed loop value ($P < 0.05$).

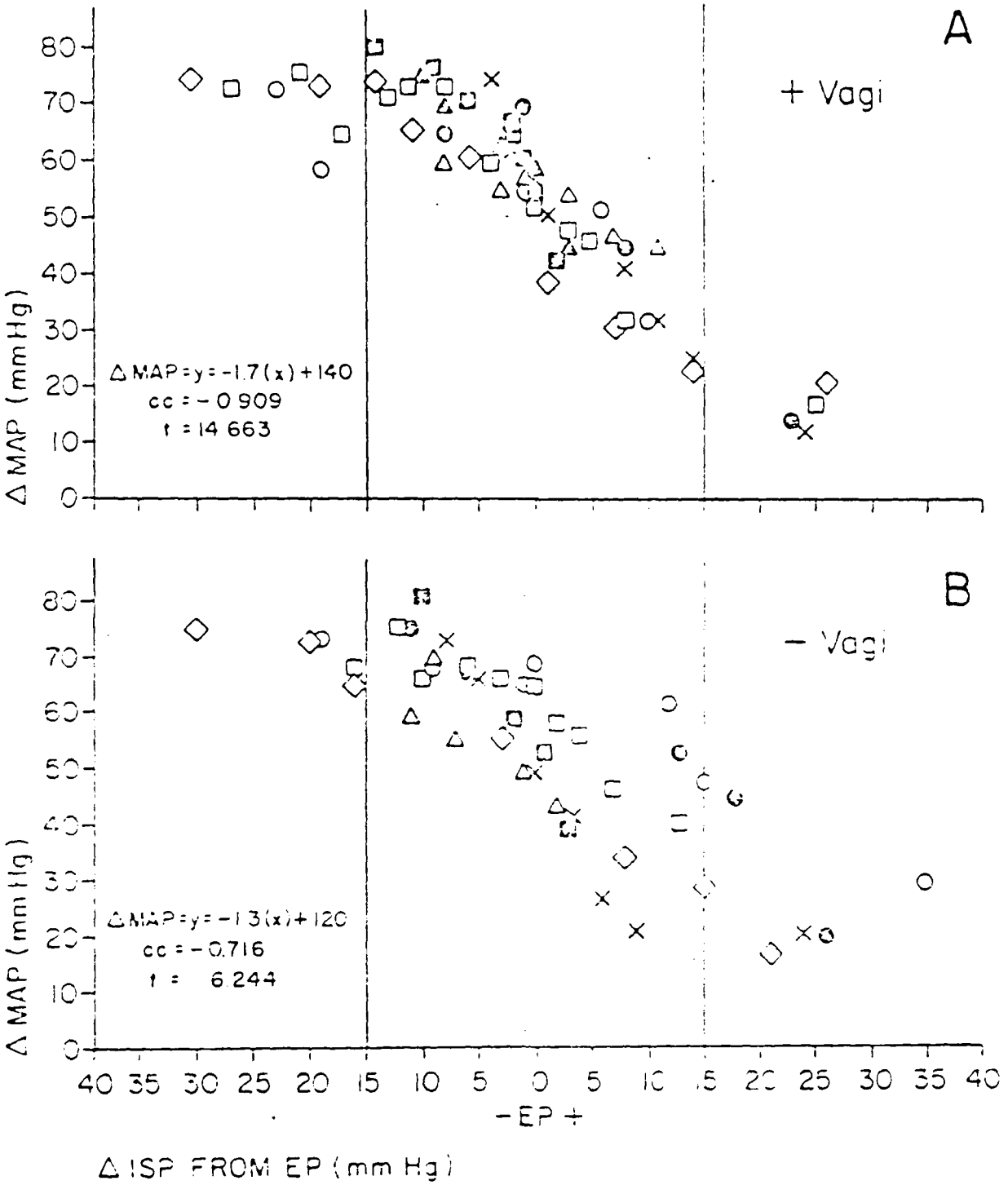
Legends

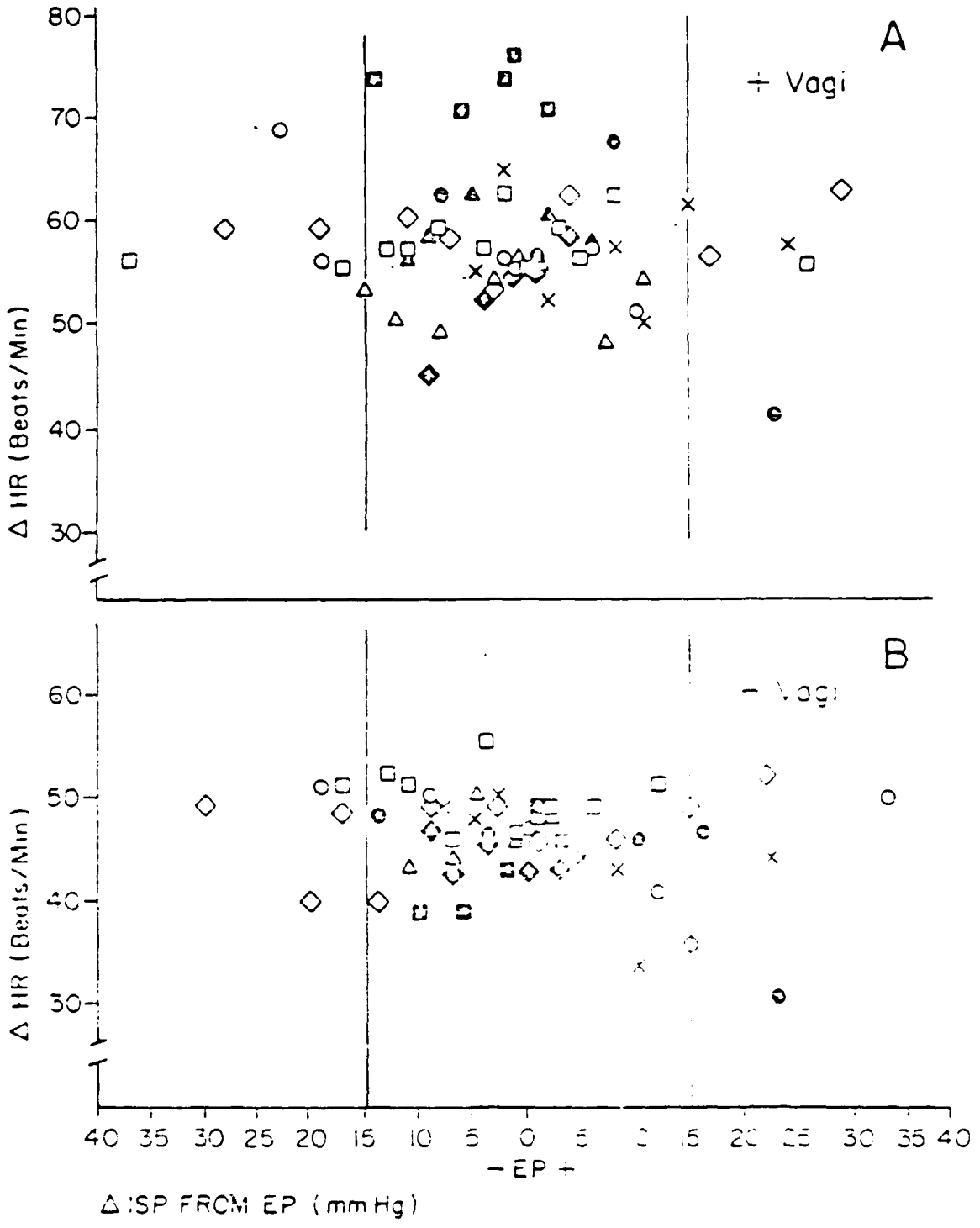
FIGURE 1: Magnitude of the fall in mean arterial pressure (ΔMAP) resulting from left aortic nerve stimulation (LANS) during control of the carotid intrasinus pressure (ISP) in eight rabbits before and seven of same after vagotomy. Each symbol represents responses of one animal. Responses were normalized between animals by setting the equilibrium pressure (EP), where ISP and MAP are equal, of each animal to zero. Regression analysis indicated a significant level of correlation of ΔMAP to ISP within ± 15 mmHg of EP both before and after vagotomy, see graphs for equations and coefficients, respectively. Significant differences in responses before and after vagotomy were not found.

FIGURE 2: Magnitude of the bradycardia (Δ HR) resulting from LANS during control of ISP in the same animals as Fig. 1. For comparison, responses were normalized in each animal and statistical analysis was the same as in Fig. 1. No significant change in ΔHR was found for individual or pooled responses either before or after vagotomy with the ISP levels tested.

FIGURE 3: Heart rate (HR), mean arterial pressure (MAP) and respective reflex cardiovascular responses (Δ HR, Δ MAP) to LANS during the conditions of circulatory inclusion (I) and exclusion (E) of the carotid sinus and vasoactive drug infusions before (● , ○) and following (▲ △) vagotomy in ten rabbits. Bars indicate SEM. Carotid sinus pressure was held at the preinfusion equilibrium pressure (EP) both before and during drug infusion. Intravenous drug infusion started in the E condition indicated by the arrows. ADH, lysine vasopressin (A, D ; NTG, nitroglycerin (N, E) and PE,

phenylephrine (C, F), *indicates significant ($P < 0.05$) difference when this value is compared to the respective (I) or (Ex-EP) control value. For further descriptions see text.


A
+ Vagi
ΔMAP=y=-1.7(x)+140
cc=-0.909
t=14.663
ΔMAP (mmHg)
B
− Vagi
ΔMAP=y=-1.3(x)+120
cc=-0.716
t=6.244
ΔMAP (mmHg)
40 35 30 25 20 15 10 5 0 5 10 15 20 25 30 35 40
−EP+
ΔISP FROM EP (mmHg)


A
+ Vagi
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Δ HR (Beats/Min)
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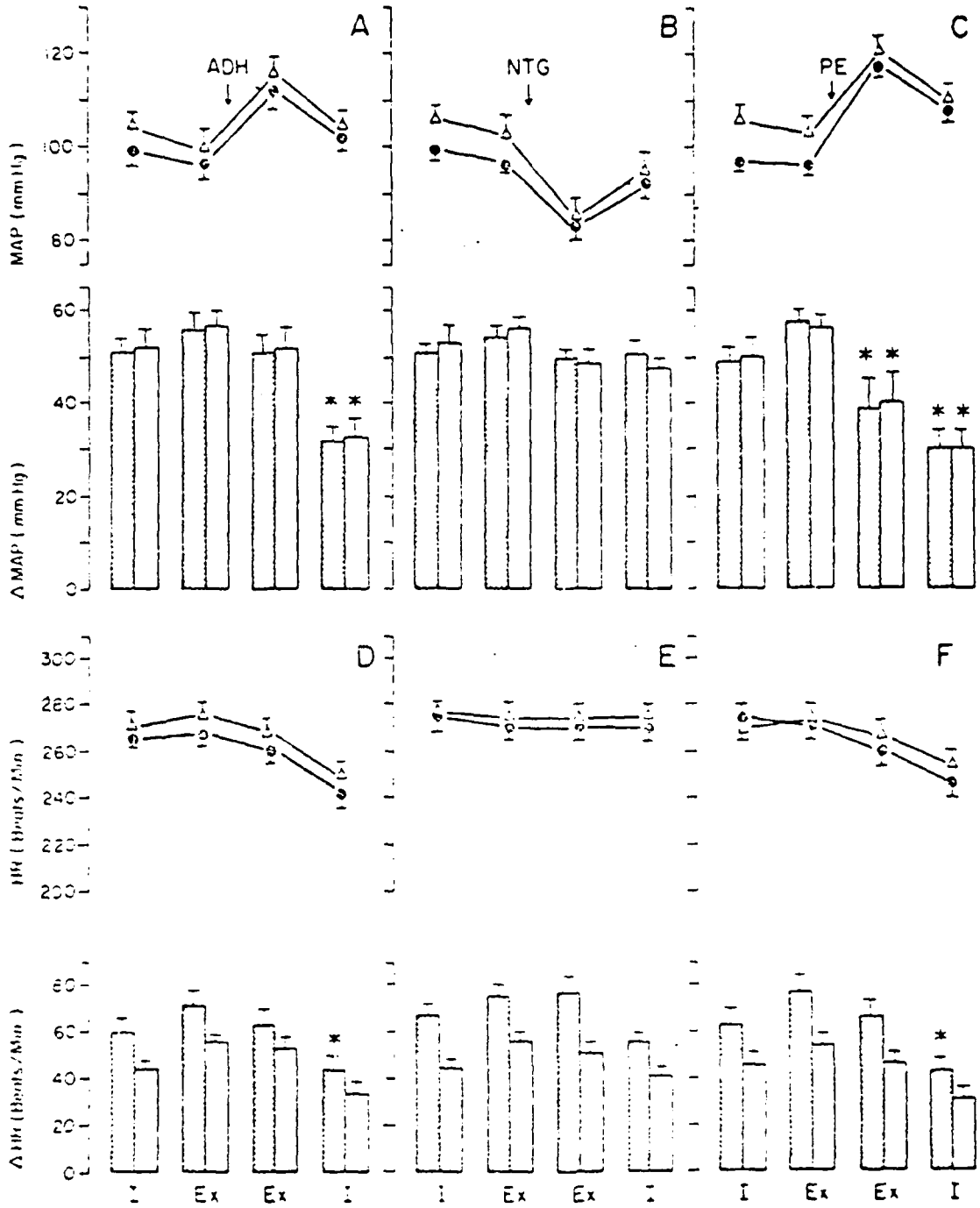
A
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